



ČESKÁ
SPOLEČNOST
EXPERIMENTÁLNÍ
BIOLOGIE ROSTLIN

bulletin ²⁰²⁵

Book of Abstracts

18th Student Days in Plant Biology CS 2025
Plant Biology CS 2025



www.csebr.cz



**Bulletin České společnosti experimentální biologie rostlin
a Fyziologické sekce Slovenské botanické společnosti**

Bulletin je vydáván s finanční podporou Akademie věd ČR.
Registrační číslo MK ČR E 13255

2025

Číslo 1/2025, ročník 2025. Vychází jednou ročně.

Toto číslo bylo dáno do výroby v srpnu 2025.

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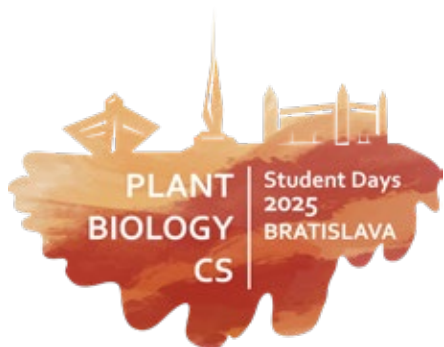
Tisk Rain, s.r.o., Otín 251, 377 01

Jindřichův Hradec

Náklad 180 výtisků

Toto číslo neprošlo jazykovou ani redakční úpravou.

ISSN 1 2 1 3-6670





Welcome words

Dear colleagues,

I am very glad to welcome you at **Plant Biology CS 2025** international conference, which is organised this time during **August 26 - 28, 2025 in Bratislava, Slovakia**. The history of this conference should follow traditional and long year lasting meetings of Czech and Slovak plant scientists (former KEBR) organized regularly in Czech Republic and Slovakia. However, in last years the conference become more international, especially after last successful meeting in České Budějovice in 2019. This time, the event is organized at the **Faculty of Natural Sciences of Comenius University in Bratislava** in close cooperation with the **Czech Society of Experimental Plant Biology** (CSEPB or ČSEBR), the **Slovak Botanical Society** (SBS) and **Plant Science and Biodiversity Centre of Slovak Academy of Sciences**. This already traditional conference is a unique opportunity for you to present your current results, get new ideas, find research partners and make a good friendships. Alongside this event, I also

welcome all participants of **Student Days of Plant Biology CS 2025**, the conference for young plant scientists, that is being held just couple of days before the main event, in **August 24 – 25, 2025** at the same place.

I would like to thank all my colleagues and organizers for their help with organizing and management of both events, as well as our invited plenary and session speakers, all session chairs and all sponsors and supporters. My thank also belongs to Dean of Faculty of Natural Sciences, Comenius University, Prof. Peter Fodor for possibility to organize these events at our faculty. My big thank also belongs to all of you, because without you and your participation we will never meet here. I hope you will find both events interesting and useful, and I wish you a pleasant stay in these days in Bratislava.

 *Marek Vaculík,*
on behalf of conference organizers



Editorial

Dear colleagues, conference participants, and friends of CSEPB and SBS,

As the president of the Czech Society of Experimental Plant Biology (CSEPB), I am happy that, after six years, we have successfully organized the Plant Biology CS conference once again. I hope this marks the beginning of a renewed tradition of having the conference, a thing that was unfortunately disrupted by the pandemic. For the future is our aim to meet at this event every four years.

I am especially pleased that this year's conference is taking place in Bratislava, hosted by our Slovak colleagues. This highlights the ongoing collaboration—and at the very least, the maintained connections—between CSEPB and SBS.

As Marek Vaculík mentioned in his previous message, this is not only the Plant Biology CS conference but also includes the preceding student conference, *Student Days in Plant Biology*. Traditionally, student conference is organised every second year, and we managed to maintain this tradition even during the pandemic. In 2021, the student conference was online. In 2023, it was organised in conjunction with the larger *Methods in Plant Sciences 2023* conference in Srní. I believe this alternating format—between “Plant Biology CS” and “Methods Days,” each accompanied by a student conference—is a sustainable, predictable, and viable model.

Combining major conferences with student events, along with (I believe) a favorable pricing policy, significantly encourages the participation of young researchers in our events. This provides to young generation

a broader overview of current developments in experimental plant biology in both the Czech Republic and Slovakia. And to the senior colleagues it gives great opportunity to meet with young successors. It also helps raise awareness of our societies among the younger generation—making them aware that we exist and that we can be of value to them.

This year, around 180 researchers are expected to attend both conferences in total, which is a decent number that suggests a wealth of high-quality contributions and exciting science. This is further enhanced by the presence of outstanding invited speakers, not only from Slovakia and Czechia. According to the program, the conference covers the full spectrum of experimental plant biology topics. Even as I write this, I am looking forward to the science that will be presented.

Finally, allow me to express my big thanks to Marek Vaculík and his colleagues from the Faculty of Natural Sciences at Comenius University for organizing this year's conference, as well as to all partners, sponsors, and colleagues from other institutions, who contributed to the successful realization of this event.

 Martin Janda,
President of the Czech Society
of Experimental Plant Biology

ORGANIZERS

Main organizer:

Department of Plant Physiology

Faculty of Natural Sciences
Comenius University in Bratislava, Slovakia



FACULTY
OF NATURAL SCIENCES
Comenius University
Bratislava



Co-organizers

Slovak Botanical Society

Dúbravská cesta 9
845 23 Bratislava, Slovakia



Institute of Botany

Plant Science and Biodiversity Centre
Dúbravská cesta 9
845 23 Bratislava, Slovakia



Czech Society of Experimental Plant Biology

Viničná 5
128 00, Praha 2, Czech Republic



Organizing committee

Marek Vaculík (FNS, IBOT, SBS)
Michal Martinka (FNS)
Alexander Lux (FNS)
Dominik Kostoláni (FNS)
Zuzana Lukačová (FNS)
Jana Kohanová (FNS)
Renáta Švubová (FNS)
Viktor Demko (FNS)
Boris Bokor (FNS)
Monika Bathóová (FNS)
Miroslava Vaculíková (IBOT)
Ján Jásik (IBOT)
Martin Janda (ČSEBR)

- SBS – Slovak Botanical Society
- FNS – Faculty of Natural Sciences, Comenius University in Bratislava
- IBOT – Institute of Botany, Plant Science and Biodiversity Centre SAS, Bratislava

PLENARY SPEAKERS – Plant Biology CS 2025



Liam Dolan

(Gregor Mendel Institute of Molecular Plant Biology, Austrian Academy of Sciences, Vienna, Austria)
Liam Dolan is a Group Leader at the Gregor Mendel Institute of Molecular Plant Biology (GMI) in Vienna, Austria. He previously served as Sherardian Professor of Botany in the Department of Biology at the University of Oxford and a Fellow of Magdalen College, Oxford from 2009 to 2021. Liam's laboratory uses genetics to discover how plants and their cells develop and evolve. The main focus has been the identification of mechanisms that control the development and differentiation of specialised plant cell types. The favorite model system is *Marchantia polymorpha*.

Available interview with Liam Dolan in *Development*:

<https://doi.org/10.1242/dev.204440>

Sources: GMI website, *Development* (Journal),
Wikipedia;



Jozef Mravec

(Institute of Genetics and Plant Biotechnology, Plant Science and Biodiversity Center, Slovak Academy of Sciences, Nitra, Slovakia)

Jozef Mravec is a group leader at the Institute of Plant Genetics and Biotechnology (SAS) in Nitra, Slovakia. He is also affiliated with the University of Copenhagen in Copenhagen, Denmark. His research focuses on plant cell wall biology, particularly on cell wall developmental dynamics and chemical biology.

Source: LinkedIn; SAS website



Viktor Žárský

(Charles University, Prague, Czech Republic)

Viktor Žárský is a Czech plant biologist, educator, and science populariser. He studied at Masaryk University in Brno and Charles University in Prague, and has worked (or continues to work) at the Institute of Experimental Botany of the Czech Academy of Sciences, Charles University, the University of Vienna, and the Max Planck Institute in Cologne. He has made significant contributions to our understanding of plant cell polarity, pollen germination, and plant morphogenesis. He was a pioneer in the study of plant exocytosis, discovering and describing the exocyst protein complex. Recently, among other things, he has been researching the colonization of the Earth by plants. In 2021, he was awarded the prestigious Donatio Universitatis Carolinae Research Grant for his scientific and pedagogical achievements.

PLENARY SPEAKER – 18th Student Days in Plant Biology CS 2025



Kateřina Macháčová

(CzechGlobe – Global Change Research Institute CAS, Brno, Czech Republic)

INVITED SPEAKERS – CSEPB AWARD



Pavel Hladík

INSTITUTE OF
EXPERIMENTAL
BOTANY AS CR, CZ



Roman Skokan

INSTITUTE OF
EXPERIMENTAL
BOTANY AS CR, CZ



Veronika Berková

MENDEL
UNIVERSITY
IN BRNO, CZ

Invited Keynote speakers – Plant Biology CS 2025



Joanna Augustynowicz

(Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Poland)



Iva Mozgová

(Institute of Plant Molecular Biology, Biology Centre CAS, České Budějovice, Czech Republic)



Maksym Danchenko

(Institute of Genetics and Plant Biotechnology, Plant Science and Biodiversity Center, Slovak Academy of Sciences, Nitra, Slovakia)



Piotr Rozpądek

(Małopolska Center of Biotechnology, Jagiellonian University in Kraków, Poland)



Michaela Havrlentová

(University of Ss. Cyril and Methodius in Trnava and NPPC – Research Institute of Plant Production, Piešťany, Slovakia)



Marko Sabovljević

(Faculty of Biology, University of Belgrade, Serbia & Faculty of Science, University of PJ Šafárik in Košice, Slovakia)



Jan Hejátko

(Mendel Centre for Plant Genomics and Proteomics, National Centre for Biomolecular Research, Faculty of Science, Masaryk University, CEITEC - Central European Institute of Technology, Brno, Czech Republic)



Jozef Šamaj

(Department of Biotechnology, Faculty of Science, Palacký University in Olomouc, Czech Republic)



Stefanie Wienkoop

(Faculty of Life Sciences, University of Vienna, Austria)

Scientific committee – 18th Student Days in Plant Biology CS 2025

Jiří Kubásek

(University of South Bohemia in České Budějovice, CZ)

Kateřina Macháčová

(CzechGlobe – Global Change Research Institute CAS, Brno, CZ)

Michal Martinka

(Faculty of Natural Sciences, Comenius University in Bratislava, SK)

Marek Vaculík

(Plant Science and Biodiversity Centre SAS; Comenius University in Bratislava; SK)

Scientific committee – Plant Biology CS 2025

Joanna Augustynowicz

(University of Agriculture in Krakow, PL)

Maksym Danchenko

(Plant Science and Biodiversity Centre SAS, SK)

Loriana Demecsová

(Plant Science and Biodiversity Centre SAS, Institute of Botany, SK)

Viktor Demko

(Comenius University in Bratislava, SK)

Liam Dolan

(Gregor Mendel Institute, Austrian Academy of Sciences, AU)

Michaela Havrlentová

(University of Ss. Cyril and Methodius in Trnava and NPPC – Research Institute of Plant Production, Piešťany, SK)

Jan Hejátko

(Masaryk University; CEITEC Brno, CZ)

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(University of South Bohemia in České Budějovice, CZ)

Alexander Lux

(Comenius University in Bratislava, SK)

Kateřina Macháčová

(CzechGlobe – Global Change Research Institute CAS, CZ)

Michal Martinka

(Comenius University in Bratislava, SK)

Jozef Mravec

(Plant Science and Biodiversity Centre SAS, SK)

Iva Mozgová

(Institute of Molecular Plant Biology, Biology Centre AS CR, CZ)

Miroslav Ovečka

(Palacký University Olomouc, CZ)

Andrej Pavlovič

(Palacký University in Olomouc, CZ)

Piotr Rozpądek

(Jagiellonian University in Kraków, PL)

Marko Sabovljević

(University of Belgrade, Serbia)

Jozef Šamaj

(Palacký University Olomouc, CZ)

Tomáš Takáč

(Palacký University Olomouc, CZ)

Marek Vaculík

(Plant Science and Biodiversity Centre SAS; Comenius University in Bratislava, SK)

Stefanie Wienkoop

(University of Vienna, AT)

Viktor Žárský

(Charles University in Prague, CZ)

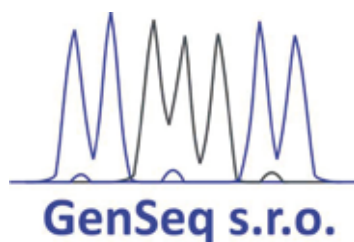
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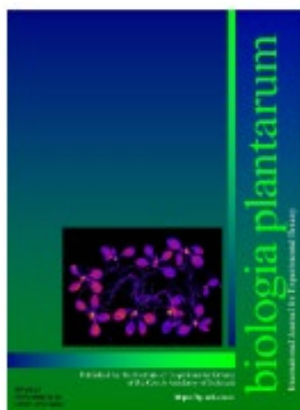
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Rada vědeckých
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Institute of Experimental
Botany of the AS CR, v. v. i.



Conference venue

Faculty of Natural Sciences Comenius University in Bratislava

Ilkovičova 6, 842 15 Bratislava, Slovakia

Lectures: Aula B1-301 (Pavilion B1)

Posters: Foyer B1 Pavilion



Additional program

Tuesday, August 26, 2025:

18:00 – 20:00 **Botanical garden excursion**

Botanical Garden of Comenius University,
Botanická 3, 841 03 Bratislava

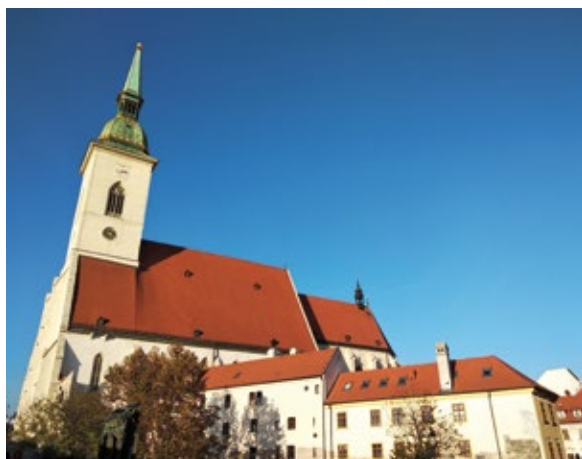
Wednesday, August 27, 2025: 17:30 – 22:30 Conference event

17:30 – 18:30 **Visit of St Martin's Cathedral and organ concert**

(St Martin's Cathedral, Rudnayovo námestie 1, 811 01 Bratislava – Staré mesto)

19:00 – 22:30 **Conference dinner**

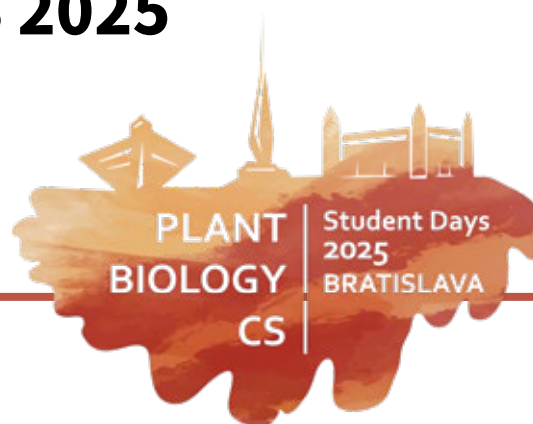
(Restaurant Bratislavský Meštiansky pivovar, Drevená 8, 811 06 Bratislava – Staré mesto)



zdroj:<https://mestianskypivovar-drevena.choiceqr.com/>

PROGRAM 18th STUDENT DAYS IN PLANT BIOLOGY CS 2025

Program – Aula B1-301



Sunday Aug 24

13:00 – 13:20 Opening

13:20 – 14:00 Invited Plenary Lecture

Kateřina Macháčová (CzechGlobe, CAS, Brno, Czech Republic)

Methane (CH₄) and nitrous oxide (N₂O) exchange of trees as a missing component in greenhouse gas balance of forest ecosystems

14:00 – 14:20 **Mohi Ud Din Atta**: Photosystems and antioxidative system of rye, wheat and triticale under Pb stress

14:20 – 14:40 **Petra Bublavá**: Interaction of light and ABA signaling in plant responses to salt stress

14:40 – 15:00 **Kateřina Cermanová**: Polyamine and ethylene biosynthesis dynamics in *Arabidopsis thaliana* and *Solanum lycopersicum*

15:00 – 16:00 Coffee and posters

16:00 – 16:20 **Sonkar Dipti**: Lipid peroxidation and protein carbonylation in plants under heat stress

16:20 – 16:40 **Katarína Heldesová**: Oligocationic peptide lys10 as a novel tool for live-cell imaging of pectin dynamics

16:40 – 17:00 **Jhonny Hernandez**: Development of spray-induced gene silencing (SIGS) approach to protect *Papaver somniferum* against fungal pathogens

17:00 – 17:20 **Chidera Anuforo**: Metal-induced resistance in *Capsicum annuum* (pepper) against *Botrytis cinerea*

18:00 – 22:00 Conference dinner (barbecue party)

Monday Aug 25

Invited lecture from CSEPB Award competition:

09:00 – 09:30 Hladík P.: Auxin quantification: From whole organs to single cells

09:30 – 09:50 **Radana Chytilová:** Plant and the beast: Host plant chemical response to oviposition by damselfly (*Lestes*)

09:50 – 10:10 **Methawin Koetkhiao:** Root border cell production of Japanese rice under salt stress

10:10 – 10:30 **Tereza Miksteinová:** The role of gibberellins in the response to osmotic stress

10:30 – 10:50 **Phatcharaporn Khamnoi:** *In vitro* culture of lotus (*Nelumbo nucifera*)

10:50 – 11:40 Coffee and posters

11:40 -12:00 **Pop Serban:** The long calmodulin7: Not just a visitor, but a plasma membrane resident

12:00 – 12:20 **Marek Súhřada:** Effect of soil additives on optimisation of phytomanagement of contaminated sites

12:20 – 12:40 **Natálie Závorková:** Interaction between blue light and abscisic acid in *Arabidopsis thaliana* under drought stress

12:40 – 14:00 Lunch

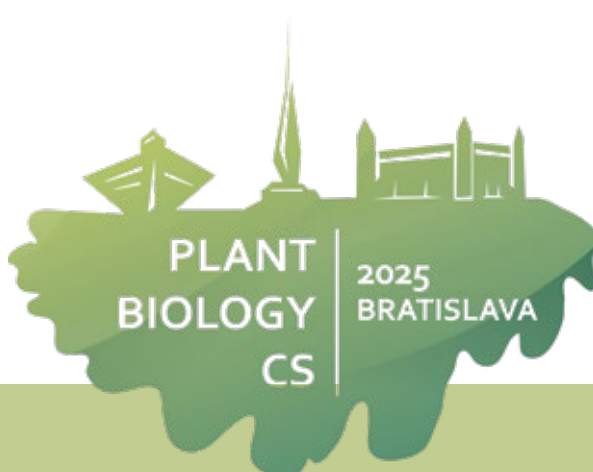
14:00 – 14:20 **Dominika Škriniarová:** Changes in germination processes of maize grains (*Zea mays* L. hybrid Ronaldino) after non-thermal plasma treatment

14:20 – 14:40 **Mohammad Umar:** Aftermath of transient drought on grain proteome of wheat

14:40 – 15:00 **Titiwut Ruamsuk:** The survey and mapping of plants for ecotourism in the surrounding areas of Tham Luang-Khun Nam Nang Non National Park (preparatory), Chiang Rai province

15:30 – 16:00 Closing ceremony and prize awards

PROGRAM PLANT BIOLOGY CS 2025



Scientific sessions:

Session 1: Cell biology, cell cycle and cell development

Session 2: Carnivorous and aquatic plants, bryophytes and lichens

Session 3: Plant microbiome, plant-organisms interactions

Session 4: Plant genetics, genomics and epigenetics

Session 5: Plant proteomics & metabolomics and plant biotechnology

Session 6: Phytohormones and root & shoot development

Session 7: Advances in microscopy and analytical techniques in plants

Session 8: Plant adaptation to abiotic stress, phytoremediation and phytotechnologies

Session 9: Plant-pathogen interaction, plant immunity & plant protection

Session 10: Crop reproduction and nutrition, biology of trees

Tuesday Aug 26

09:00 – 09:15 Opening

09:15 – 10:00 Invited Plenary lecture 1

Viktor Žárský (Charles University, Prague CZ)

Functional diversification of exocyst complex subunits, plant terrestrialization and polysporangiate origin of land plants

10:00 – 10:30 Coffee break

10:30 – 11:45 Session 1 - Cell biology, cell cycle and cell development

Chair: Iva Mozgová (Biology Centre CAS, České Budějovice, CZ)

10:30 – 10:45 **Viktor Demko**: Genetic analyses of plant calpain DEK1 in *Physcomitrium patens* uncover multiple layers of its essential role during growth and development

10:45 – 11:00 **David Honys**: Keeping cool under pressure: How eIF3 safeguards pollen fertility and thermotolerance

11:00 – 11:15 **Ivan Kulich**: ARO-dependent channelosomes are essential for variety of rapid cellular responses, from auxin to abscisic acid, across all land plants

11:15 – 11:30 **Thanakon Chaichana**: Suspension establishment and heat shock response of golden gardenia (*Gardenia sootepensis* Hutch.)

11:30 – 11:45 **Rajdeep Ghosh**: The role of class I formins at plasmodesmata

12:00 – 13:30 Lunch

13:30 – 14:30 Session 2: Carnivorous and aquatic plants, bryophytes and lichens

Chair: Andrej Pavlovič (*Palacký University in Olomouc, CZ*)

13:30 – 14:00 INVITED LECTURE

Marko S. Sabovljević: Bryophyte conservation physiology

14:00 – 14:15 **Andrej Pavlovič:** The diversity of digestive systems in carnivorous plants

14:15 – 14:30 **Monika Danchenko:** Molecular cloning and expression profile of a unique protease from carnivorous sundew

14:30 – 16:00 Session 3: Plant microbiome, plant-organisms interactions

Chair: Martin Janda (*University of South Bohemia in České Budějovice, CZ*)

14:30 – 15:00 INVITED LECTURE

Piotr Rozpądek: The importance of microorganisms in plant adaptation to the environment

15:00 – 15:15 **Barbora Jindřichová:** Does fungal infection increase the palatability of oilseed rape to insects?

15:15 – 15:30 **Weronika Kosowicz:** The role of glucosinolates in mutualistic interaction between plants and their endophytic microorganisms

15:30 – 15:45 **Viktor Nagy:** The role of arbuscular mycorrhizal fungi in regenerative agriculture under the conditions of the Czech Republic

15:45 – 16:00 **Rafał Ważny:** The role of seed endophytes in plant adaptation to toxic metals

16:00 – 17:30 Coffee & posters

16:15 – 18:15 Plenary meeting of ČSEBR – Aula B1-301

INVITED LECTURE FROM CSEPB AWARD COMPETITION:

16:30 – 16:45 Veronika Berková: The fungus *Acremonium alternatum* enhances salt stress tolerance by regulating host redox homeostasis and phytohormone signalling

16:45 – 17:00 Roman Skokan: Pre-hormonal nature of auxin unravelled in streptophyte algae

18:00 – 20:00 Botanical garden excursion (welcome drink & snacks)

Wednesday Aug 27

08:30 – 09:15 Invited Plenary Lecture 2

Jozef Mravec (*Plant Science and Biodiversity Center, SAS, Nitra, SK*)

How novel cell wall-directed probes helped discover some unexpected biological phenomena

09:15 – 11:15 Session 4: Plant genetics, genomics and epigenetics

Chair: Viktor Demko (*Comenius University in Bratislava, SK*)

09:15 – 09:45 INVITED LECTURE

Iva Mozgová: Unravelling the contribution of polycomb repression to metabolic and developmental transitions in plants

09:45 – 10:00 **Jana Balarynová:** Seed coat-specific polyphenol oxidase expression results in hilum pigmentation

10:00 – 10:15 **Jan Bartoš:** The maize B chromosome exerts an influence on the transcriptome throughout plant development

10:15 – 10:30 **Fatima Cvrčková:** Why so many? Interpreting natural variability and gene expression to unravel function in large gene families
10:30 – 10:45 **Ján Jásik:** Plant synaptotagmins
10:45 – 11:00 **Tereza Přerovská:** Unveiling plant telomere diversity: Lessons from *Allium cepa* TRB proteins
11:00 – 11:15 **Petr Smýkal:** Domestication as convergent and parallel evolution – comparative analysis of chickpea, lentil, pea and common beans for two key domestication traits – pod dehiscence and seed dormancy

11:15 – 11:45 Coffee break

11:45 – 13:00 Session 5: Plant proteomics & metabolomics and plant biotechnology

Chair: Tomáš Takáč (Palacký University in Olomouc, CZ)

11:45 – 12:15 INVITED LECTURE

Maksym Danchenko: Aquatic plants in Chernobyl are susceptible to pathogens

12:15 – 12:30 **Tomáš Takáč:** Unveiling novel proteins governing oxidative stress response in *Arabidopsis*
12:30 – 12:45 **Marcela van Loo:** Drought sensitivity and metabolic adaptations to drought stress: A case study in seedlings of a non-model tree species
12:45 – 13:00 **Mykola Borysyuk:** Comparative transcriptome/proteome analyses reveal key pathways responsive to manganese stress in aquatic plant *Spirodela polyrhiza*

13:00 – 14:30 Lunch

14:30 – 15:30 Session 6: Phytohormones and root & shoot development

Chair: Alexander Lux (Comenius University in Bratislava, SK)

14:30 – 15:00 INVITED LECTURE

Jan Hejátko: Cytokinin/ethylene crosstalk in the control of root growth

15:00 – 15:15 **Samia Belaidi:** Role of the AUXIN RESPONSE FACTOR5 and microRNA390 in embryogenic transition in *Arabidopsis thaliana*
15:15 – 15:30 **Pavel Krupař:** Unraveling the relationship between root elongation and cell wall pH

15:30 – 16:30 Coffee & posters

17:30 – 22:30 Conference event (St Martin's Cathedral – visit and organ concert) and conference dinner (Restaurant: Bratislavský Meštiansky pivovar, Drevená)

Thursday Aug 28

9:00 – 09:45 Invited Plenary Lecture 3

Liam Dolan (GMI, Austrian Academy of Sciences, Vienna, AT)

Developing meristems *de novo*

09:45 – 11:00 Session 7: Advances in microscopy and analytical techniques in plants

Chair: Miroslav Ovečka (Palacký University in Olomouc, CZ)

09:45 – 10:15 INVITED LECTURE

Jozef Šamaj: Advanced microscopy for plants

10:15 – 10:30 **Aleš Pěnčík:** Comparative metabolite profiling approach reveals the complexity of auxin metabolism across plant species

10:30 – 10:45 **Chao Zhang:** *In situ* comparison of abiotic and biotic induced phytohormone changes using mass spectrometry imaging

10:45 – 11:00 **Michal Karady:** Advancing plant metabolic research - validated approach for simultaneous ethylene, phytohormone and polyamine detection

11:00 – 11:30 Coffee break

11:30 – 13:30 Session 8: Plant adaptation to abiotic stress, phytoremediation and phytotechnologies

Chair: Marek Vaculík (Comenius University in Bratislava, SK)

11:30 – 12:00 INVITED LECTURE

Joanna Augustynowicz: *Callitriche* sp. – between a model study and application

12:00 – 12:15 **Loriana Demecsová:** Nitric oxide sustains root surface redox activity and growth during flooding stress in barley root tip

12:15 – 12:30 **Alexander Lux:** Structural aspects of plant reactions to stress

12:30 – 12:45 **Emmanuel Opoku:** Differences in the production of root exudates and physiological responses of C3 and C4 crops to drought stress and nitrogen fertilization

12:45 – 13:00 **Peter Paľove-Balang:** Nitrogen and other abiotic factors affecting isoflavonoid and flavonoid production in *Lotus* sp.

13:00 – 13:15 **Helene Robert Boisivon:** Impact of heat waves on seed development in *Arabidopsis thaliana* and *Brassica napus*

13:15 – 13:30 **Marek Vaculík:** Effect of metals and metalloids on growth of various species and ecotypes from *Salicaceae* family

13:30 – 14:45 Lunch

14:45 – 16:00 Session 9: Plant-pathogen interaction, plant immunity & plant protection

Chair: Michal Martinka (Comenius University in Bratislava, SK)

14:45 – 15:15 INVITED LECTURE

Stefanie Wienkoop: From pathogen resistance to seed resilience: The role of flavonoids and microbial partners in pea plants

15:15 – 15:30 **Martin Janda:** Pattern-triggered immunity in *Papaver somniferum*

15:30 – 15:45 **Tetiana Kalachova:** Battlefield - plant: exploring the dynamics of tripartite interactions between plants, bacteria and bacteriophages

15:45 – 16:00 **Hana Leontovyčová:** Hormonal crosstalk in fungal pathogenicity: auxin, cytokinin and salicylic acid of *Leptosphaeria maculans*

16:00 – 17:00 Session 10: Crop reproduction and nutrition, biology of trees

Chair: Loriana Demecsová (Plant Science and Biodiversity Center, SAS, Bratislava, SK)

16:00 – 16:30 INVITED LECTURE

Michaela Havrlentová: Plants as nutritional pillars: understanding, optimizing, and reprogramming crop composition for human benefit

16:30 – 16:45 **Zuzana Kovaliková:** Biochemical responses of sweet cherry to repeated water deficit

16:45 – 17:00 **Péter Májer:** Effect of natural-based biostimulants on poppy yield parameters

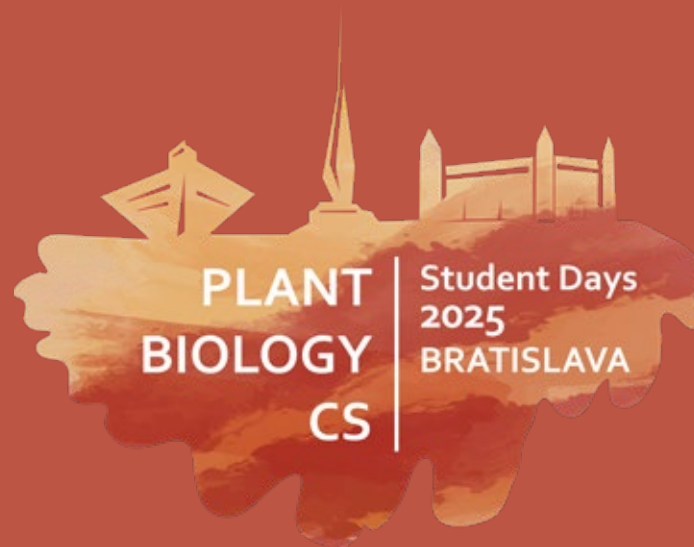
17:00 – 17:30 Coffee break

17:30 – 18:00 Closing ceremony and prize awards



24th–25th August 2025

18th Student Days in Plant Biology CS 2025



Abstracts

ORAL LECTURES

METHANE (CH₄) AND NITROUS OXIDE (N₂O) EXCHANGE OF TREES AS A MISSING COMPONENT IN GREENHOUSE GAS BALANCE OF FOREST ECOSYSTEMS

PLENARY
INVITED
TALK

Macháčová Kateřina

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Key words: flux, forest, greenhouse gas, soil, tree

Methane (CH₄) and nitrous oxide (N₂O) are important greenhouse gases, which contribute to global climate change. Soils are well-known as substantial sources and sinks of CH₄ and N₂O. Besides soils, trees can also emit or take up these greenhouse gases under certain conditions. However, the role of trees in forest ecosystem CH₄ and especially N₂O exchange is still far from being understood.

In the invited presentation, Katerina will introduce the processes and pathways connected to CH₄ and N₂O exchange in forest ecosystems, the contribution of trees to the forest greenhouse gas flux dynamics, and limitations and challenges of the current research. After giving an overview of the measurement techniques for determination of CH₄ and N₂O fluxes from soils, and tree stems and leaves, she will present interesting team`s research outputs, investigating natural exchange and flux dynamics of CH₄ and N₂O in different tree species (stems and leaves), cryptogams, soils and forest ecosystems along a broad geographical and climatic gradient.

Based on the results presented, we will see that tree species of boreal, temperate and tropical zone can substantially contribute to ecosystem greenhouse gas exchange. The determination of the role of trees in CH₄ and N₂O exchange of various forest ecosystems is therefore of high importance for correct estimations of forest CH₄ and N₂O budgets and therefore of global greenhouse gas flux inventories.

Acknowledgement

Many thanks to a large team of researchers involved in the presented studies. Co-authors of all individual studies will be acknowledged in the presentation. The research was supported by the Ministry of Education, Youth and Sports of CR within the LU - INTER-EXCELLENCE II (2022 - 2029) program [grant number LUC23162] and project AdAgriF - Advanced methods of greenhouse gases emission reduction and sequestration in agriculture and forest landscape for climate change mitigation [CZ.02.01.01/00/22_008/0004635].

AUXIN QUANTIFICATION: FROM WHOLE ORGANS TO SINGLE CELLS

INVITED
TALK- CSEPB
AWARD

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Key words: auxin, cell, LC-MS/MS, metabolism

Auxins play a central role in plant development, yet their quantification and metabolic profiling remain challenging due to their low abundance and the complexity of plant matrices. My research addresses this by developing advanced LC-MS/MS-based analytical methods for the highly sensitive detection and quantification of auxins and other phytohormone metabolites, from whole organs down to subcellular compartments.

My Ph.D. research led to the identification and characterization of previously unknown catabolites of indole-3-acetic acid (IAA) and phenylacetic acid (PAA), significantly expanding our understanding of irreversible auxin metabolism. Through comparative profiling across four model species—*Arabidopsis*, maize, pea, and wheat—we uncovered striking interspecies differences in auxin metabolic preferences. These findings challenge the long-held concept of a conserved auxin inactivation strategy across angiosperms. Furthermore, we conducted the most comprehensive analysis of PAA metabolism to date, revealing shared enzymatic machinery between PAA and IAA pathways. This suggests a coordinated regulation of these two major auxins in plants. To push the boundaries of phytohormone research, we integrated fluorescence-activated cell sorting (FACS) with optimized dispersive solid-phase extraction. This methodological breakthrough allows for precise hormone quantification in samples as small as 50,000 cells or 200,000 organelles. This capability enables cell-type-specific profiling of hormone metabolism in *Arabidopsis thaliana* root tips, generating high-resolution phytohormone maps across distinct root cell types. Collectively, these findings offer novel insights into the diversity of auxin metabolism and provide powerful tools for dissecting hormone function with cellular resolution. This work opens new approaches for future research in plant developmental biology, stress adaptation, and crop improvement.

Acknowledgement

This work was supported by the project TowArds Next GENeration Crops, of the ERDF Programme 630 Johannes Amos Comenius [grant number CZ.02.01.01/00/22_008/0004581].

METAL-INDUCED RESISTANCE IN *CAPSICUM ANNUUM* (PEPPER) AGAINST *BOTRYTIS CINEREA*

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Key words: fungal pathogen, pepper, metabolites, metalloproteins, micronutrients

This study is aimed at investigating the molecular mechanisms, by which Zn and Cu mediate enhanced induced resistance in *Capsicum annuum* L. (pepper) challenged with the generalist necrotrophic fungal pathogen *Botrytis cinerea*. Previous reports have shown a potential of Zn priming for efficient defence in the same patho-system (Kuvelja et al., 2024), but much of the mechanisms is still to be explored. The findings can help to reduce the unsustainable use of chemical pesticides by priming plants with low, environmentally relevant concentrations of Cu and Zn that are not toxic for the environment. This is in contrast to high Cu concentrations in fungicides used in vineyards, which accumulate to toxic levels.

To identify the physiological response to Zn and Cu priming and pathogen infection, time-resolved experiments were done in hydroponics, ranging from Zn and Cu-deficiency (0.08 μ M Zn, 0.02 μ M Cu), optimal (1 μ M Zn, 0.3 μ M Cu) and priming, not-growth-limiting, 5 μ M Zn and 1 μ M Cu. The preliminary data on metal distribution measured in vivo by benchtop micro-XRF will be correlated with the changes in chlorophyll fluorescence kinetics determined by direct OJIP imaging and the level of infection, as well as total element content. The results will further be integrated with metalloproteomics and mRNA sequencing, while isotope-assisted untargeted metabolite profiling by LC-HRMS/MS will be done to find priming-induced and pathogen-induced metabolites. Finally, to validate the role of the candidate metabolites and/or Zn- and Cu-binding enzymes in the immunity of metal-primed pepper plants, genetic manipulation (knockout) and physiological analyses of mutant plant lines will be combined with in vitro and exogenous treatment assays.

Reference

Kuvelja, A., Morina, F., Mijovilovich, A., Bokhari, S. N. H., Konik, P., Koloniuk, I., & Küpper, H. (2024). Zinc priming enhances *Capsicum annuum* immunity against infection by *Botrytis cinerea*—From the whole plant to the molecular level. *Plant Science*, 343, 112060.

Acknowledgement

The study is funded by the FWF-GACR grant 25-19190L, by the Ministry of Education of the Czech Republic with co-financing from the European Union (grant KOROLID, CZ.02.1.01/0.0/0.0/15_003/0000336), COST Action CA 19116 “Trace metal metabolism in plants-PLANTMETALS, and Czech Academy of Sciences (RVO 60077344).

PHOTOSYSTEMS AND ANTIOXIDATIVE SYSTEM OF RYE, WHEAT AND TRITICALE UNDER Pb STRESS

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Key words: antioxidative capacity, cereals, lead, photosynthesis; protein, Triticeae

Lead (Pb²⁺) pollution in the soil sub-ecosystem has been a continuously growing problem due to economic development and ever-increasing anthropogenic activities across the world. In this study, the photosynthetic performance and antioxidant capacity of *Triticeae* cereals (rye, wheat and triticale) were compared to assess the activities of antioxidants, the degree of oxidative damage, photochemical efficiency and the levels of photosynthetic proteins under Pb stress (0.5 mM, 1 mM and 2 mM Pb (NO₃)₂). Compared with triticale, Pb treatments imposed severe oxidative damage in rye and wheat. In addition, the highest activity of major antioxidant enzymes (SOD, POD, CAT, and GPX) was also found to be elevated. Triticale accumulated the highest Pb contents in roots. The concentration of mineral ions (Mg, Ca, and K) was also high in its leaves, compared with rye and wheat. Consistently, triticale showed higher photosynthetic activity under Pb stress. Immunoblotting of proteins revealed that rye and wheat have significantly lower levels of D1 (photosystem II subunit A, PsbA) and D2 (photosystem II subunit D, PsbD) proteins, while no obvious decrease was noticed in triticale. The amount of light-harvesting complex II b6 (Lhcb6; CP24) and light-harvesting complex II b5 (Lhcb5; CP26) was significantly increased in rye and wheat. However, the increase in PsbS (photosystem II subunit S) protein only occurred in wheat and triticale exposed to Pb treatment. Taken together, these findings demonstrate that triticale shows higher antioxidant capacity and photosynthetic efficiency than wheat and rye under Pb stress, suggesting that triticale has high tolerance to Pb and could be used as a heavy metal-tolerant plant.

Reference

Mohi Ud Din A, Mao HT, Khan A, Raza MA, Ahmed M, Yuan M, Zhang ZW, Yuan S, Zhang HY, Liu ZH, Su YQ. Photosystems and antioxidative system of rye, wheat and triticale under Pb stress. *Ecotoxicology and Environmental Safety*. 2023 Jan 1;249: 114356.

Acknowledgement

This work was supported by Applied Basic Research Program of Sichuan Province (Projects No: 2020YJ0410), Sichuan Science and Technology Program (Projects No: 2022YFH0068) and the National Natural Science Foundation of China (Projects No: 41967033 and 32102759). We are especially grateful to Tevanu Berthier (Nanjing Agricultural University) and Shozeb Haider (University College London) for their critical reading and language polishing during the revision of this manuscript. The Presenting Author is thankful to Department of Experimental Plant Biology, Faculty of Science and Biology Center, Czech Academy of sciences for their support.

INTERACTION OF LIGHT AND ABA SIGNALING IN PLANT RESPONSES TO SALT STRESS

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Key words: abscisic acid, light, salt stress, tomato

In recent years, research focused on plant stress is becoming more and more important due to deteriorating environmental conditions. Salt stress has emerged as a major agricultural problem, limiting plant growth and development. It is a primary factor causing soil degradation, leading to reduced soil fertility and crop productivity. Understanding the interplay between light and the abscisic acid (ABA) signaling pathway is pivotal in uncovering how plants adapt to environmental stress conditions. ABA is a key hormone in plant response to stress and its level is altered by light conditions. This fact indicates that light can play a significant role in plant adaptation to stress.

This study focuses on a tomato (*Solanum lycopersicum* L.) as a model organism to investigate the mechanisms behind plant responses to salt stress. Specifically *hp1* (*high pigment 1*) mutant, characterized by a defect in the DDB1 (UV-DAMAGED DNA BINDING 1) protein, which is involved in photomorphogenesis, stress responses and stability in plants.

Experiments with 7-day-old seedlings revealed that DDB1 deficiency in *hp1* mutants enhances light sensitivity through intensified light signaling. This altered light signaling promotes higher expression of the HY5 gene in mutant plants. HY5 is a key transcription factor in the regulation of light-mediated responses. Further, defect in DDB1 protein significantly affected ABA biosynthesis and signaling, particularly influencing the expression of the *ABI5* gene, which acts as a convergence point between the light and ABA pathways. These findings suggest that light and ABA closely cooperate in the plant's response to stress.

The results of this study provide insight into the mechanisms linking light and ABA signaling pathway during salt stress. This knowledge can contribute to a better understanding of how plants respond to stress conditions and may have potential applications in improving stress tolerance in crops.

COMBINED EFFECT OF ELEVATED ATMOSPHERIC CO₂ CONCENTRATION AND NITROGEN AVAILABILITY ON THE METABOLISM AND PHYSIOLOGY OF *CALAMAGROSTIS VILLOSA*

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POSTER

Key words: elevated atmospheric CO₂, nitrogen availability, secondary metabolism

Changes in atmospheric CO₂ concentration directly influence plant physiology and growth. To predict the responses of natural plant communities to future increases in CO₂, it is crucial to understand how species and ecosystems respond to elevated CO₂ and their ability to exploit newly created niches. While much of the research in forest ecosystems has focused on trees, understory communities received only limited attention, despite their essential role in maintaining species diversity, habitat stability, and ecosystem processes.

This study investigates the responses of *Calamagrostis villosa*, a grass species representative of understory vegetation, to elevated (EC, 700 ppm) and ambient (AC, 400 ppm) CO₂ concentrations, with varying nitrogen availability. The experiment was conducted in experimental lamellar domes at the Bílý Kříž site in the Beskydy Mountains. Photosynthetic characteristics were measured using an open gasometric system, while samples were collected for broad-spectrum metabolomics and elemental analyses.

Results showed that elevated CO₂ increased photosynthetic CO₂ assimilation rates, with N availability having no significant effect. However, reduced N availability increased the variability in CO₂ assimilation. Elevated CO₂ also enhanced water use efficiency, with slight stimulation observed under higher N availability. In contrast, N availability significantly reduced the C:N ratio, while elevated CO₂ had less of an effect. Phenolic acids, such as vanillic and syringic acid, generally decreased with higher N availability, whereas the effect of elevated CO₂ depended on N availability. Other phenolic compounds were slightly stimulated by elevated CO₂, with nitrogen availability having a bigger effect. These findings indicate the complex interaction effects of elevated CO₂ and nitrogen availability on plant physiology.

Acknowledgement

This research was financially supported by the Internal Grant Agency of MENDELU (AF-IGA2022-IP-044) and the AdAgriF project (CZ.02.01.01/00/22_008/0004635).

POLYAMINE AND ETHYLENE BIOSYNTHESIS DYNAMICS IN *ARABIDOPSIS THALIANA* AND *SOLANUM LYCOPERSICUM*

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Key words: abiotic stress, ethylene, HPLC-MS/MS, polyamines

Polyamines are ubiquitously present in all living organisms. In plants, together with phytohormone ethylene, their metabolism plays a crucial role in plant stress and ontogenesis. As these pathways are directly interconnected, their simultaneous evaluation can significantly impact our understanding of their core mechanisms.

We have therefore developed a novel and validated liquid chromatography-tandem mass spectrometry based method, enabling quantification of polyamines, amino acids, and ethylene precursors, and evaluated differences in the responses of model plants *Arabidopsis thaliana* and *Solanum lycopersicum* to abiotic stresses based on key metabolite levels.

Our analysis revealed distinct metabolic responses between *Arabidopsis* and tomato, highlighted by species-specific differences in polyamine metabolism and ethylene precursors dynamics. Drought and salinity stresses triggered fundamentally different metabolic adjustments, with drought consistently inducing higher metabolite levels and spermine showing stress-specific responses. Additional experiments with *Arabidopsis* mutants affected in ethylene synthesis and arginine metabolism pathways further confirmed the interconnected nature of these metabolic networks and their responses to pathway perturbations. For all approaches, *Arabidopsis* displayed more pronounced metabolic fluctuations compared to tomato. These results provide direct insights into contrasting metabolic plasticity and the interconnected roles of polyamines, amino acids, and ethylene precursors in plant responses and adaptations.

Acknowledgement

Presented work was supported by The Czech Science Foundation (GAR) via 20-25948Y junior grant, by Internal Grant Agency of Palacky University (IGA_PrF_2025_019) and by the "Biorefining and circular economy for sustainability" (TN02000044) grant.

LIPID PEROXIDATION AND PROTEIN CARBONYLATION IN PLANTS UNDER HEAT STRESS

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Key words: heat stress, malondialdehyde, photosystem II, reactive oxygen species

Under environmental conditions, plants are exposed to various abiotic and biotic stress factors. High temperature (HT) stress triggers the overproduction of reactive oxygen species (ROS), which commonly cause the oxidation of lipids and proteins. Lipid peroxidation and the generation of reactive aldehydes, such as malondialdehyde (MDA), a secondary products of lipid peroxidation. MDA can form covalent adducts with photosystem II proteins. The spatial distribution of MDA-protein adducts in *Arabidopsis* leaves shows that MDA-protein adducts are located in the chloroplasts, uniformly spread out over the thylakoid membrane. thereby impairing the function of vital complexes, such as photosystem II (PSII). This study examined MDA-mediated protein modifications in *Arabidopsis thaliana* wild-type (WT) and lipoxygenase1-2 (*LOX1-2*) mutant plants exposed to 40°C for 0 h and 24 h. Western blot analysis using primary anti-MDA antibody revealed increased MDA-protein adducts in thylakoid membranes after 24 h of heat stress, particularly in WT plants. While decreasing in *LOX1-2* 24 h. Co-immunoprecipitation using anti-PsbP and Lhcb1 antibody showed specific MDA modification at the PsbP and Lhcb1 proteins. HPLC-based quantification from the leaves confirmed elevated levels of total MDA under heat stress. These findings suggest that MDA selectively modifies specific PSII proteins. This modification may contribute to PSII dysfunction and degradation under prolonged heat stress.

Acknowledgement

A grant funded this work. IGA_PrF_2025_028 entitled "Current research topics in molecular and general biophysics" of Palacky University. We thank Dr. Marek Rc for technical support.

OLIGOCATIONIC PEPTIDE LYS10 AS A NOVEL TOOL FOR LIVE-CELL IMAGING OF PECTIN DYNAMICS

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Key words: cell wall, fluorescence imaging, homogalacturonan, peptide probe

The plant cell wall is a dynamic matrix, continuously remodelled during growth, differentiation and in response to environmental cues. Homogalacturonan (HG) – the most abundant pectic polysaccharide – plays a central role in this process because its degree of methyl-esterification directly modulates wall mechanics and cell-to-cell adhesion. Yet visualising HG dynamics in living tissue has remained difficult as traditional immunolabeling has been proven largely ineffective for such applications.

To address this gap, we present Lys10 – a decamer of L-lysine tagged with carboxyfluorescein – as a small oligocationic probe that binds de-esterified HG via ionic interaction. In dot-blot assays, Lys10 bound strongly to polygalacturonic acid, in comparison to esterified pectins, β -glucan pachyman, and other controls. Control staining with decameric arginine or glutamic acid probes yielded significantly lower to negligible signal, underscoring the unique compatibility of Lys10 structure and chemistry with de-esterified HG. *In silico* analysis further supported a high-affinity interaction between Lys10 and its target and molecular dynamics simulation will be presented in this work as well.

Fluorescence microscopy confirmed that Lys10 stains cell walls both *in vitro* (*Arabidopsis* stem cross-sections) and *in vivo*. In live *Arabidopsis thaliana* Col-0 roots, Lys10 readily penetrated all tissue layers and labelled cell walls throughout the organ. Employing Lys10 for time-lapse confocal imaging, we successfully visualized HG's dynamic changes and distribution during root hair elongation, revealing localized labeling patterns likely corresponding to sites of pectin methylesterase activity.

Together, these findings establish Lys10 as efficient probe for visualising de-esterified homogalacturonan in living plants, opening a straightforward route to real-time studies of pectin dynamics and cell-wall remodelling.

Acknowledgement

Funded by NextGenerationEU through the Recovery and Resilience Plan for Slovakia under project No. 09I03-03-V02-00005, grant of Slovak Academy of Sciences IM-2021-23 and VEGA 2/0162/24.

DEVELOPMENT OF SPRAY-INDUCED GENE SILENCING (SIGS) APPROACH TO PROTECT PAPAVER SOMNIFERUM AGAINST FUNGAL PATHOGENS

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Key words: dsRNAs, fungal pathogens, plant protection, poppy

Papaver somniferum (poppy) is part of the neglected crops, but has an important role in the pharmaceutical industry as it is used to produce alkaloids used as painkillers. Poppy seeds are used in food industry being highly consumed on countries such as the Czech Republic. As other plant species poppy is susceptible to be infected and killed by pathogens and pests. However the knowledge about their specific pathogen and infection process are still unraveled. This sheds light on the problem of controlling this pathogen and pests. Fungal plant pathogens such as *B. cinerea*, *S. sclerotiorum*, and *Alternaria* species cause diseases in poppy cultivars. These fungal pathogens are well studied because they can infect a wide range of plants. For these fungal pathogens spray-induced gene silencing (SIGS) strategy was developed and we copied the strategy to test it also in poppy. On the other hand, *A. papavericola*, is a specialist with specific host which is poppy. Importantly, and *A. papavericola* is one of the most harmful pathogens affecting poppies. *A. papavericola* has not been studied at all and it is unknown how this fungus is able to infect poppies so effectively. This gap in our knowledge poses a problem when it comes to developing innovative strategies to protect poppy. In our research, we established *A. papavericola*-*P. somniferum* pathosystem to study SIGS as a strategy to protect poppy cultivars. We have discovered that some candidate genes can be successfully silenced in *A. papavericola* and lead to reduce infection in poppy

leaves. Beyond SIGS, our overall aim is to study how fungal infection process occurs, to develop novel strategies to protect poppy against fungal pathogens with particular focus on *A. papavericola*.

Acknowledgement

We would like to thank for financial support from MEYS Inter-Excellence II, Inter-COST project nr. LUC23146.

PLANT AND THE BEAST: HOST PLANT CHEMICAL RESPONSE TO OVIPOSITION BY DAMSELFLY (*LESTES*)

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Key words: cytokinins, oviposition, plant defense, stress phytohormones, UHPLC-MS/MS

Plants respond to oviposition by herbivorous insects by triggering a signal cascade similar to that which is well-known following injury to plant tissue. The eggs of herbivorous insects, as immobile and seemingly inactive stages, have been intensively studied over the last three decades when considering plant-herbivore interactions. However, it is still not known how a plant responds to oviposition by an animal that does not potentially threaten it but instead helps it figuratively by consuming herbivorous insects. Dragonflies are powerful predators that consume hundreds of thousands of those insects. From an evolutionary point of view, the question arises as to whether the plant adapts its response to the oviposition of an insect predator such as a dragonfly, potentially protecting the plant from insects living in the plant tissue. Our preliminary case study was performed to verify methods and it proved that monitoring of hormonal response is feasible and repeatable. The study analyzed the phytohormonal response of the plant common rush (*Juncus effusus*) to oviposition by the damselfly (*Lestes sponsa*) through quantitative analysis of stress phytohormones and cytokinins by ultra-high-performance liquid chromatography-tandem mass spectrometry. However, individual hormones and their crosstalk need more molecular and genetic data for a better understanding of the precise involvement of these pathways.

ROOT BORDER CELL PRODUCTION OF JAPANESE RICE UNDER SALT STRESS

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Key words: Japanese rice, root border cells, salt stress

Root border cells (RBCs) at the root tip act as a biological barrier, facilitating root growth through soil while protecting the root meristem and mitigating salinity stress, which can harm plant growth, photosynthesis, and protein synthesis, thereby reducing crop yield and quality. This study analyzed RBC formation in Japanese rice (*Oryza sativa* L. ssp. *japonica* cv. Koshihikari). Seeds soaked in water for 24 hours, drained, and incubated in darkness produced the highest number of RBCs, with germination occurring within 4–6 days. Under salinity stress, RBC production appeared to decline at NaCl concentrations above 60 mM compared to the control and 10 mM NaCl treatments. RBCs exhibited three distinct shapes: spherical (in the root cap), rectangular (in the division zone), and elongated (in elongation and differentiation zones). Despite limited research on RBCs in monocots like rice, this study highlights their potential role in salinity stress tolerance. Given the economic importance of Koshihikari rice in Thailand and globally, these findings provide a foundation for further research into improving salinity stress tolerance in this crucial crop.

THE ROLE OF GIBBERELLINS IN THE RESPONSE TO OSMOTIC STRESS

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Key words: biosensor, drought, gibberellin, osmotic stress

Drought is considered as one of the most prevalent and damaging stresses, which influences germination, plant development and crop production. Due to the challenges of studying the effect of drought on roots in the soil environment, we currently use osmotic stress to impose water restriction *in vitro*.

Phytohormones play an essential role in early seedling development and its adaptation to stress. We are interested in the involvement of the growth hormone gibberellin (GA) and its interaction with abscisic acid (ABA) in the stress response. GA is an important hormone which promotes germination, growth of developing tissues as well as flowering. In addition, in response to stress GA plays a major role in the balance between growth and survival, for example in the redistribution of growth between leaves to roots that occurs in response to water limitation. ABA acts primarily to control stomatal behaviour and modifying root architecture to enhance adaptation.

We are using *Arabidopsis thaliana* seedlings to better understand the involvement of GA signaling in the early response to osmotic stress and in the communication between roots and shoots. The stress is induced *in vitro* using polyethyleneglycol and we are monitoring its effect on expression of GA and ABA metabolism genes using qRT-PCR and gene reporters, while the contribution of the genes to the stress response is investigated using metabolism mutants. To determine the effect on GA content and distribution with high spatial resolution we are using the Gibberellin Perception Sensor (GPS2).

Acknowledgement

Funding: IGA_PrF_2025_019; European Regional Developmental Fund to project "Towards Next Generation Crops" No. CZ.02.01.01/00/22_008/0004581(TANGENC).

THE ROLE OF ARBUSCULAR MYCORRHIZAL FUNGI IN REGENERATIVE AGRICULTURE UNDER THE CONDITIONS OF THE CZECH REPUBLIC

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Key words: arbuscular mycorrhiza, regenerative agriculture, soil health, no-till, cover crops

Arbuscular mycorrhizal fungi (AMF) are crucial for sustainable agriculture and soil health. However, their contribution to regenerative farming—particularly under Central European climatic and agronomic conditions—remains insufficiently understood. Regenerative practices such as no-till farming combined with cover cropping offer great potential for long-term soil carbon sequestration, but the specific responses of AMF under these conditions require further investigation.

This study evaluated the impacts of different soil management strategies on AMF colonisation in winter wheat (*Triticum aestivum*) roots at two long-term experimental sites near Banín, where field trials have been conducted since 2019. A total of 20 pre-selected plots were assessed, comprising treatments of no-till with cover crops and conventional tillage without cover crops, each under mineral or organic fertilization regimes. Five representative root systems per plot were analysed for AMF colonization.

Our results show that AMF colonisation was significantly higher in no-till plots with cover crops. This is likely due to reduced soil disturbance and the continuous propagation of AMF during the off-season, both of which favour AMF persistence and inoculum preservation. In contrast, conventional tillage combined with wind erosion appears to facilitate spore translocation, potentially destabilizing AMF communities.



POSTER

These findings highlight the importance of reduced tillage and cover cropping in enhancing AMF networks, which could be pivotal for improving soil resilience and advancing sustainable agricultural systems.

Acknowledgement

This research was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic through the AdAgriF project (CZ.02.01.01/00/22_008/0004635).

IN VITRO CULTURE OF LOTUS (*NELUMBO NUCIFERA*)

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Key words: Shoot apical meristem, Plant stem cell, Embryo, Secondary metabolite

This study aimed to establish an optimized protocol for tissue and cell culture of Lotus (*Nelumbo nucifera* Gaertn.), focusing on aseptic germination, shoot apical meristem culture, and callus induction. Aseptically germinated lotus embryos were used as explants and subjected to six treatments (20 samples/ treatment). The result show that the highest survival rate (85%) was achieved by soaking the explants in 15% (v/v) of Clorox (6% hypochlorite) for 20 minutes. Embryos of lotus were cultured on Murashige and Skoog (MS) basal medium which can enhance the shoot development within 14 days. The sterilized plantlet production was maintained in a plant tissue culture room for 6 weeks before transferring to liquid MS medium supplemented with 0.3 mg/L TDZ and 8 mg/L NAA. After 2 weeks in liquid media, the multiple leaves emerged, and significant leaf and root proliferation was observed during 3–4 weeks. Comparison of callus induction from embryos and meristematic tissue was used of 4-week-old lotus tissues (including leaves and internodes) that cultured on MS medium containing 2 mg/L 2,4-D and 0.1 mg/L TDZ. During callus induction, the lotus leaf tissues expanded within 7–14 days and small calluses formed within 30 days. Callus induction from sterilized embryos was performed on solid MS medium supplemented with 7.5 mg/L NAA and 0.1 mg/L TDZ for 14 days, followed by transfer to solid MS medium containing 2 mg/L 2,4-D and 0.1 mg/L TDZ for 30 days. As a result, a large and prominent embryo-derived callus was observed. For suspension induction, callus aged 6–8 weeks was scraped and cultured in liquid MS medium with 2 mg/L 2,4-D and 0.1 mg/L TDZ for one week and single cells of lotus were found. We will use the calluses to produce a cell suspension culture focused on plant-stem cell and secondary metabolite analysis in the future, aiming for food and drug development as well as a model of aquatic plant cells for gene transformation.

Acknowledgement

This project was supported by University of Phayao and Demonstration School University of Phayao Science Classroom in University Affiliated School (SCIUS).

THE LONG CALMODULIN7: NOT JUST A VISITOR, BUT A PLASMA MEMBRANE RESIDENT

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Key words: Ca²⁺ signaling; rapid cell signaling, stress response

Calmodulins (CaMs) are ubiquitous and highly conserved calcium (Ca²⁺) sensor proteins that regulate diverse cellular processes such as morphogenesis, stress responses, and hormone signalling. In *A. thaliana*, all CaM isoforms are considered redundant due to their high sequence similarity and overlapping expression patterns. They predominantly localise to the cytoplasm and are capable of shuttling to the nucleus. However, while CaMs have the propensity for targeting plasma membrane (PM) proteins, none of them have been observed to have an intrinsic PM association. Here we report a CaM7 splicing

variant with a C-terminal extension to the canonical form, the 'long Calmodulin 7' (loCaM7), which contains a large polybasic stretch and a prenylation site that is identical to proteins with established PM-localisation, such as AtROP6. By screening transient expressions of fluorescently tagged constructs we confirmed this variant does localise to the PM. Additionally, we have found that loCaM7 is mostly expressed in the root and upregulated in response to various stresses, such as cold. Our aim is to investigate this functionally unexplored splice variant and to elucidate its role within Ca²⁺ signaling in the context of Arabidopsis root stress response, as well as other potential physiological processes.

Acknowledgement

This project is supported by the Czech Science Foundation grant Nr. 25-16449S and by European Union, Horizon Europe, project MOLIPeC, ID 101087030. We acknowledge the core facility LMH, the BC CAS supported by the MEYS CR (LM 2023050 Czech-Biolmaging).

EFFECT OF SOIL ADDITIVES ON OPTIMISATION OF PHYTOMANAGEMENT OF CONTAMINATED SITES

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Key words: biochar; mycorrhiza; silicon; soil additives

The success of phytomanagement in contaminated areas depends not only on the selection of suitable plant species, but also on the use of effective soil amendments to enhance plant resilience and facilitate pollutant uptake or stabilization. Our research on English plantain (*Plantago lanceolata*) grown in a substrate from nickel ore processing demonstrated that mycorrhizal fungi significantly improve plant nutrient uptake, stress tolerance, and contaminant immobilization, making them a crucial component of remediation strategies. The application of mycorrhizal fungi led to a marked increase in photosynthetic rate, biomass production, and chlorophyll content. Moreover, plants grown in the presence of mycorrhizal fungi showed no signs of oxidative stress.

In contrast, two other soil amendments used in our study, silicon and biochar, showed only limited effectiveness in supporting phytomanagement efforts. In all samples cultivated without mycorrhizal fungi, the addition of silicon and biochar had a negative impact on photosynthesis, biomass production, and chlorophyll content. Although these amendments may offer benefits for soil structure and pollutant adsorption, their role in optimizing plant-based remediation remains uncertain. Further research is necessary to develop effective soil amendment strategies that support sustainable ecological restoration of old mining and metal processing sites.

Acknowledgement

This work was carried out as part of projects supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic (VEGA), project no. VEGA 1/0472/22, and projects supported by the Slovak Research and Development Agency, project no. APVV SK-TW-24-0008 and APVV-17-0164.

CHANGES IN GERMINATION PROCESSES OF MAIZE GRAINS (ZEA MAYS L. HYBRID RONALDINO) AFTER NON-THERMAL PLASMA TREATMENT

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Key words: germination, hydrolytic enzymes, maize, non-thermal plasma

Treatment of seeds and grains with non-thermal plasma generated from ambient air (DCSBD - diffuse coplanar surface barrier discharge) has a positive effect on germination process and plant development. The plasma has a decontamination effect on the seed and grain surfaces, and also activates the hydrolytic enzymes during the germination process. The main hydrolytic enzymes are

the subject of our interest. Rapid activation of hydrolytic enzymes and mobilization of storage substances is essential for the initiation of germination processes. This work is aimed at studying the dynamics of gene expression and subsequent activation of hydrolytic and antioxidant enzymes.

Acknowledgement

I would like to thank to my supervisor doc. Mgr. Renáta Švubová, PhD. The projects that are financing our work APVV-21-0147, VEGA 1/0334/25, UK/1092/2025.

SUSPENSION ESTABLISHMENT AND HEAT SHOCK RESPONSE OF GOLDEN GARDENIA (*GARDENIA SOOTEPENSIS HUTCH.*)

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POSTER

Key words: cell suspension culture, *Gardenia sootepensis* Hutch., heat stress response, plant tissue culture

Gardenia sootepensis Hutch., known as golden gardenia, is a medicinal evergreen tree native to Southeast Asia with notable antimicrobial, anti-inflammatory, cytotoxic, and antioxidant properties. Despite its pharmacological value, little is known about its cellular responses to abiotic stress, particularly heat stress. This study aimed to develop in vitro culture protocols and investigate heat tolerance in suspension cells derived from leaf explants. Callus induction was successfully achieved using Murashige and Skoog (MS) medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D; 0.5–2.0 mg/L) and kinetin (Kn; 0.1 or 0.2 mg/L), yielding a 100% induction rate. Calli formed with 0.5–1.0 mg/L 2,4-D were friable and olive-green, while 2.0 mg/L produced browning. Suspension cultures established with 0.5–1.0 mg/L 2,4-D and 0.1 mg/L Kn showed enhanced cell proliferation and biomass accumulation. Heat shock experiments revealed that *G. sootepensis* suspension cells tolerated exposure to 55°C (extracellular medium temperature of $46.7 \pm 0.10^\circ\text{C}$) for 5 minutes without visible structural damage. These results demonstrate the species' potential for in vitro propagation and its initial resilience to heat stress. Further research is needed to refine culture conditions, assess long-term stability and genetic fidelity, and deepen understanding of stress response mechanisms.

Acknowledgement

This research was supported by University of Phayao, and Demonstration School, University of Phayao.

THE SURVEY AND MAPPING OF PLANTS FOR ECOTOURISM IN THE SURROUNDING AREAS OF THAM LUANG-KHUN NAM NANG NON NATIONAL PARK (PREPARATORY), CHIANG RAI PROVINCE

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Key words: forest surveys; GIS; national parks; NDVI; satellite images

This research focuses on surveying and mapping plant species for Ecotourism in the surrounding areas of Tham Luang-Khun Nam Nang Non National Park (Preparatory), Chiang Rai Province, using Geographic Information System (GIS) technology and the Normalized Difference Vegetation Index (NDVI) analysis from Landsat 8 satellite images. The study evaluates biodiversity and vegetation density in the area, which includes mixed deciduous and montane forests that are rich in natural resources and biodiversity. Field surveys were conducted along four nature trails, using GPS data to record the geographic

coordinates of each plant species. The collected data was analyzed and mapped with ArcGIS software for accuracy. The plant species were verified through academic databases and field validation. The NDVI analysis highlighted vegetation health and greenness levels. NDVI values, calculated using wavelengths of 0.63–0.70 μm (red) and 0.7–1.1 μm (near-infrared), range from -1 to 1. Results showed that NDVI values in 2024 (densely vegetated areas 55%) were higher than in 2015 (densely vegetated areas 42%), indicating improved vegetation density. This study developed a vegetation map and a Normalized Difference Vegetation Index (NDVI) map using the ArcGIS program, which is highly significant for long-term natural resource conservation planning. The findings can support the development of eco-trails and sustainable forest conservation plans. Moreover, the data can serve as a guideline for restoring degraded forest areas affected by climate change and human activities in the future.

Acknowledgement

We express our profound gratitude to the personnel of Tham Luang - Khun Nam Nang Non Forest Park for their invaluable facilitation, provision of expert knowledge, geospatial data, and on-site assistance crucial to our research, survey, and mapping endeavors.

AFTERMATH OF TRANSIENT DROUGHT ON GRAIN PROTEOME OF WHEAT

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Key words: contrasting cultivars, LC-MS, storage proteins, *Triticum aestivum*, water shortage

Drought stress frequency and intensity have significantly increased globally because of climate change, which has been particularly detrimental to crop productivity, including the widely cultivated bread wheat (*Triticum aestivum*). Stress during the reproductive stage has the most potent effect on yield decline. Herein, we evaluated the impact of moderate drought during flowering on grain quality across 2 contrasting cultivars—the sensitive cultivar Chyhyrynka and the tolerant cultivar Sofiia Kyivska. Proteins were isolated with single-step detergent-assisted extraction, digested with trypsin, and processed with liquid chromatography-mass spectrometry. We quantified 5,433 proteins in mature grains and revealed 728 differentially abundant proteins across genotypes and drought treatment. According to the principal component analysis, genotype contributed more to protein accumulation variance than drought treatment, with a distinct grouping of tolerant and sensitive cultivars. The protein profiling determined that seed storage proteins, such as glutenin, gliadin, cupin type-1, serpin, and globulin isoform 1, accumulated similarly after drought in both cultivars. Furthermore, several proteins involved in metabolic reactions were significantly depleted upon stress. Of note, the total grain yield declined considerably in the sensitive genotype. Next, we will focus on redox proteome alteration in flag leaves under water shortage and subsequent recovery at the reproductive stage as the most critical for yield formation. The discoveries will reveal molecular markers and pathways essential for developing drought-resilient wheat to improve crop yield in the face of progressing climate change.

Acknowledgement

The study was supported by the project VV-MVP-24-0368.

INTERACTION BETWEEN BLUE LIGHT AND ABSCISIC ACID IN *ARABIDOPSIS THALIANA* UNDER DROUGHT STRESS

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Key words: abscisic acid, blue light, drought, phototropins

Drought stress significantly affects plant growth, with abscisic acid (ABA) playing a key role in stomatal closure to minimize water loss. Blue light (BL), perceived by phototropins, also regulates stomatal movement, but its interaction with ABA signaling beyond this function remains unclear. This study explores the role of phototropins (PHOT1, PHOT2) in ABA-mediated drought responses using *Arabidopsis thaliana* mutants.

In vivo drought stress experiments showed that the 6 weeks old *phot2* mutant plants are less drought tolerant under both WL and BL. Under white light, drought-stressed *phot2* plants exhibited lower ABA levels than wild-type (*gl-1*), while BL exposure reversed this effect. Gene expression analysis suggests this increase in the ABA level is linked to upregulated *BG1*, involved in ABA deconjugation. Additionally, *NCED3* gene for a key ABA biosynthesis enzyme, and gene *RD29b* for a drought stress marker, were induced under stress conditions.

In vitro assays with polyethylene glycol (PEG) 8000 confirmed that phototropins, mainly PHOT2, influence root growth under drought stress. *In vitro* assays with ABA showed that *phot2* mutant displayed reduced sensitivity to ABA-induced root growth inhibition across different light conditions. However, ABA metabolism remained largely unchanged in phototropin mutants, suggesting that phototropins, particularly PHOT2, modulate ABA sensitivity (signalling) rather than biosynthesis.

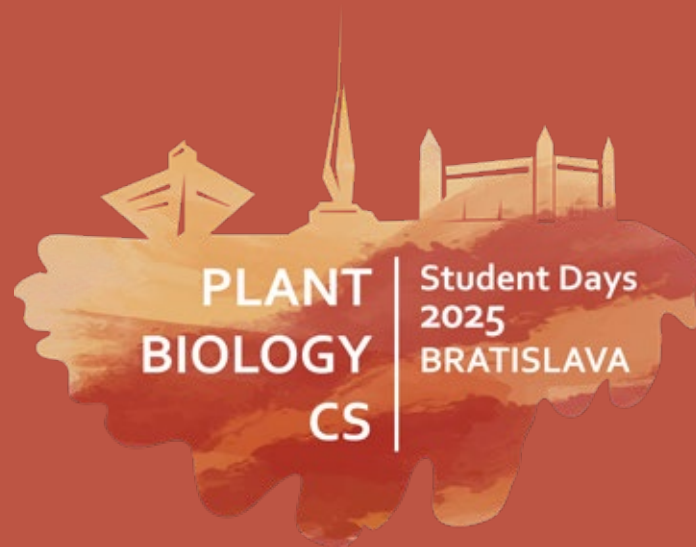
These findings highlight the role of phototropins in plant drought adaptation and suggest a complex interplay between BL and ABA. Prepared studies of chloroplast movement will hopefully broaden our understanding of this mechanism.

Acknowledgement

This work was supported by IGA_PrF_2025_019 grant and Visegrad Fellowship 62510023.

24th–25th August 2025

18th Student Days in Plant Biology CS 2025



Posters

CAN SILICON SUPPLEMENTATION STRENGTHEN SORGHUM'S DEFENSE AGAINST APHIDS? INSIGHTS INTO MORPHOLOGICAL AND BIOCHEMICAL RESPONSES

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Key words: aphids, oxidative stress, plant defence, silicon, *Sorghum bicolor*

Aphids are among the most destructive pests that affect agricultural crops worldwide, with chemical insecticides remaining the primary method of control. Due to the environmental risks of insecticides, such as soil degradation and harm to beneficial insects, sustainable alternatives are needed. Silicon shows promise in improving plant resistance to various stresses. This study aimed to evaluate the efficacy of silicon supplementation in enhancing the defence mechanisms of *Sorghum bicolor* against aphid infestation. The plants were cultivated in a silicon-enriched soil substrate and subjected to controlled aphid exposure. After the cultivation morphological and production parameters were assessed to calculate tolerance indices. To investigate the biochemical response, we quantified superoxide radicals and measured the activity of superoxide dismutase, a key antioxidant enzyme involved in mitigating oxidative stress. While silicon at the tested concentration did not markedly enhance tolerance to aphid infestation, the morphological and physiological responses, especially observable in the younger shoot organs, suggest substantial potential. These findings support further exploration of silicon in sustainable pest management strategies.

Acknowledgement

The project was supported by VEGA1/0745/20, UK/360/2023 and UK/3197/2024 grants.

A PROTEO-TRANSCRIPTOMIC APPROACH TO CHARACTERIZE THE HEAT STRESS RESPONSE OF ARABIDOPSIS THALIANA SEEDS

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Key words: alternative splicing, *Arabidopsis thaliana*, heat stress, multiomics, seed

Here, I present how we used proteomics and transcriptomics methods to decipher the heat stress response of *Arabidopsis thaliana* seeds at two different developmental stages—two days after pollination and three days after pollination. We examined differential expression to identify genes whose protein and transcript abundances changed, followed by calculating the correlation between changes in protein and transcript levels after heat stress. Additionally, we analyzed genes that were alternatively spliced at the transcriptomic level and searched for peptides that would confirm the translation of these transcript isoforms at the proteomic level.

The results show that genes differentially expressed ($\log_2FC > 1$ and $\text{adj. } P. \text{ val} < 0.05$) at both the proteomic and transcriptomic levels are in the minority. Most differentially expressed genes were found to be regulated at either the proteomic or the transcriptomic level, but not both. Despite this, our data show a strong correlation—greater than 0.7—between proteomic and transcriptomic changes. We also demonstrated that a few peptides were detected at the proteomic level, supporting the conclusion that alternative splicing has a measurable impact on protein abundance. The Gene Ontology terms enriched after heat treatment were similar at both transcriptomic and proteomic levels, including terms such as heat stress response and heat stress tolerance. However, the terms enriched among alternatively spliced genes were distinct and did not include heat stress response or tolerance. This suggests that alternative splicing and heat stress are both important for the heat stress

response but affect different sets of genes involved in plant growth and development. Furthermore, we observed that even a small developmental difference—just 24 hours between the two stages—resulted in significant variation in differentially expressed and spliced genes. This indicates that the heat stress response differs notably between these two developmental stages in the seed.

Acknowledgement

Unravelling the involvement of PRP8 in mRNA splicing during embryogenesis and in seed thermoresponse, MU code GA22-29717S.

USING PROXIMITY LABELING TO IDENTIFY INTERACTING PARTNERS OF PLANT CALPAIN DEK1 IN *PHYSCOMITRIUM PATENS*

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Key words: calpain protease, cell fate, plant development, protein-protein interaction

The DEFECTIVE KERNEL 1 (DEK1) protein is a main regulator of cell fate and development in plants. The protein consists of a 23-spanning transmembrane domain, a juxtamembrane Linker domain, and C-terminal Calpain protease acting as an effector of DEK1. Despite its essential functions during embryogenesis and seed endosperm development, the genuine molecular targets of DEK1 still remain obscure. In our lab, we use the model plant *Physcomitrium patens* to dissect the molecular function of DEK1. We utilized *proximity labeling* approach to capture proteins in vicinity of the full-length DEK1 as well as its Calpain domain *in vivo*. We generated *P. patens* lines with inserted biotin ligase TurboID in the wild type *DEK1* locus, and in *DEK1* deletion mutant with and without Calpain domain. Here, we present a design of the experiment and initial molecular and phenotypic characterization of the obtained transgenic lines that will be used for further proteomics analyses.

Acknowledgement

This work was supported by the Slovak Research and Development Agency grant APVV-21-0227. We thank Yasin Dagdas from Gregor Mendel Institute of Molecular Plant Biology GmbH, Vienna for providing the TurboID vectors, Alain Shumbusho, Matej Zámečník, and Rebecca Horváthová for initiating this work.

DISTRIBUTION AND CONTENT OF SCYTONEMIN IN COLONIES OF ANTARCTIC CYANOBACTERIA *NOSTOC SP.* IN RESPONSE TO SOLAR RADIATION

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Key words: Antarctic cyanobacteria, Raman spectroscopy, scytonemin, solar radiation

Some cyanobacterial species produce special compounds in their sheath to prevent damage by intense solar radiation, especially those that grow in extreme environments. We have collected samples of *Nostoc sp.* colonies from James Ross Island, Antarctica and studied the differences in the amount of scytonemin content in various parts of the colony and their changes under higher radiation conditions – elevated PAR and UV doses. To determine the amount of scytonemin, we used Raman spectroscopy. This non-destructive method allowed us to study the pigment content dynamics after the exposition to elevated radiation doses and

during recovery. The bands of the scytonemin molecule were assessed at ~ 1598 , ~ 1552 , ~ 1321 , and ~ 1172 cm^{-1} . The scytonemin was also studied as a ratio to carotenoids based on the Raman bands at ~ 1598 cm^{-1} (scytonemin, $\nu(\text{CCH})$ aromatic ring quadrant stretch) and the carotenoid band in the region between 1515 - 1525 cm^{-1} ($\nu(\text{C}=\text{C})$ stretching). The first results confirmed that the upper side of the colony, usually more exposed to sunlight, showed higher scytonemin content than the bottom. Moreover, the spatial visual color differences in the colony correspond to a different amount of scytonemin and carotenoids, with significantly enhanced signal of scytonemin detected at brown zones, compared to green zones. The content of scytonemin after radiation exposure and physiological activity patterns during recovery will be presented and discussed.

EFFECT OF SALICYLIC ACID ON *ARABIDOPSIS THALIANA* METABOLISM AND CUTICLE PROPERTIES

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Key words: immunity, photosynthesis, plant cuticle, respiration, salicylic acid

Plants have evolved a sophisticated interconnected defence system against pathogens, traditionally divided into passive mechanical and chemical defences, and active immunity. Plant immunity is triggered when the molecules associated with pathogens are recognised. An important player in plant immunity is the salicylic acid (SA) phytohormone and its signalling pathway. However, the components of passive defence are not as passive as one might think; they change, renew, and evolve during the life of the plant organs. Triggered immunity is energetically demanding and can affect the development of passive defence systems. In this work, we focus on the effect of endogenously modulated concentration of SA on plant metabolism, and the development and properties of plant mechanical barriers. We used a collection of *Arabidopsis thaliana* mutants with modulated SA concentration. Using LI-COR, we measured their photosynthetic and respirational activity. By two different methods: calcofluor white staining and water loss, we compared their cuticle permeability. Using GC-MS, we also measure composition of cuticular waxes and cutin matrix. The data obtained contribute to a better understanding of how SA inhibits plant growth and how active immunity interacts with passive defence mechanisms.

Acknowledgement

This work was supported by the GAJU grant 027/2023/P.

TEMPORAL DYNAMICS OF ANTIOXIDANT ENZYMES DURING EARLY DARK-GROWN DEVELOPMENT IN MAIZE

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Key words: antioxidant enzymes, circadian regulation, dark-grown development, maize seedlings, *Zea mays* L.

Circadian clocks are internal timekeeping mechanisms that enable organisms to anticipate and adapt to predictable daily environmental changes. In plants, these endogenous rhythms coordinate various physiological processes, including antioxidant defense. In this study, we investigated time-of-day-dependent changes in antioxidant enzyme activities and peroxidase

gene expression in maize (*Zea mays* L.) seedlings during the earliest stages of development under continuous darkness. Seedlings were cultivated in Petri dishes and sampled on the 3rd, 4th, and 5th day after germination at regular intervals over a 24-hour cycle. Growth parameters and enzymatic activities of guaiacol peroxidase (G-POX), ascorbate peroxidase (APX), and catalase (CAT), along with total soluble phenol content, were quantified spectrophotometrically. Additionally, temporal expression patterns of selected peroxidase genes were assessed using real-time PCR. These findings provide insight into the temporal regulation of antioxidant systems in dark-grown maize seedlings and suggest that early developmental processes may be influenced by endogenous timekeeping mechanisms, even in the absence of external light cues.

Acknowledgement

I would like to express my sincere gratitude to my supervisor, Zuzana Lukačová, for her invaluable guidance, support, and encouragement throughout this research. This work was supported by the Comenius University grant no. UK/1166/2025 and by the Slovak Research and Development Agency under contract Nr. APVV-17-0164, and VEGA1/0472/22.

PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF DROUGHT TOLERANCE DURING PEA SEED GERMINATION

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Key words: antioxidant, enzyme activity, conductivity, osmotic stress, pea, seeds

Seed germination is a critical developmental phase that determines seedling establishment and is highly susceptible to environmental stress, particularly water deficit. To investigate natural variation in drought resilience, 185 *Pisum sativum* landraces were evaluated under optimal hydration and osmotic stress conditions induced by polyethylene glycol (PEG 6000). Germination traits, including rate and final germination percentage, were quantified. Based on performance under stress, landraces were classified into three tolerance clusters. Notably, a subset exhibited stable germination under osmotic stress, indicative of enhanced physiological adaptation. A significant negative correlation was detected between seed size and drought tolerance, suggesting a potential trade-off between seed resource allocation and desiccation resilience. Membrane integrity during early imbibition was assessed via electrical conductivity, while oxidative stress responses were characterized through biochemical assays. Total antioxidant capacity (TAC) was elevated in tolerant landraces under stress, correlating with enhanced protection mechanisms. Total protein content varied among accessions, with higher levels generally associated with improved metabolic activity and germination performance. Lipid peroxidation was reduced in tolerant genotypes, indicating lower oxidative membrane damage. Furthermore, the activities of two key antioxidant enzymes - superoxide dismutase (SOD) and peroxidase (POD) - were higher in tolerant landraces, suggesting their role in mitigating oxidative stress during germination. Collectively, the integration of physiological, membrane stability and antioxidant profiles provided a comprehensive understanding of drought tolerance mechanisms during early developmental stages.

Acknowledgement

Work is supported by TANGENC (CZ.02.01.01/00/22_008/0004581) OP JAK project.

THE OVERLOOKED SIGNAL: REVEALING THE ROLE OF DIHYDROZEATIN IN PLANT REPRODUCTION

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Key words: cytokinin metabolism, dihydrozeatin, plant reproduction, seed development, zeatin reductase

Cytokinins are essential plant hormones that play a key role in regulating almost all aspects of plant growth and development, including reproduction. While the major forms of isoprenoid cytokinins, such as *trans*-zeatin and isopentenyladenine, have been extensively studied, much less is known about dihydrozeatin (DHZ) – a structurally distinct isoprenoid cytokinin characterized by a reduced side chain.

DHZ has been consistently observed to accumulate in plant reproductive structures, such as flowers, fruits, and seeds, across various species. Its levels often peak during critical developmental stages, including floral bud opening, seed filling, maturation and desiccation, when the levels of *trans*-zeatin decline. Despite its confirmed biological activity and clear association with reproductive tissues, both the biosynthetic origin and developmental function of DHZ remain largely unexplored. My research is focused on elucidating the biosynthesis of DHZ and its role in plant reproductive development, with a particular focus on seed formation and maturation. To achieve this, cytokinin profiling using UHPLC-MS/MS was conducted in dry seeds of several crop species to identify developmental stages associated with DHZ accumulation. Several candidate oxidoreductase genes – putative zeatin reductases – were found to be upregulated during DHZ accumulation. Their enzymatic activity was tested via transient expression in *Nicotiana benthamiana*. In a complementary experiment, we confirmed that *Arabidopsis thaliana* is capable of DHZ production when cultured on medium supplemented with *trans*-zeatin. These results support a model in which DHZ is synthesized via enzymatic reduction of *trans*-zeatin during key reproductive transitions.

By expanding our understanding of DHZ biosynthesis and function, this research contributes to a more complete view of cytokinin-mediated regulation in plant reproduction. In the long term, uncovering DHZ function could inform new strategies for improving seed quality and yield stability in crops facing variable environmental conditions.

Acknowledgement

The work was supported from the project TowArds Next GEneration Crops, reg. no. CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes Amos Comenius.

IDENTIFICATION OF THE PUTATIVE PATHWAY OF SALICYLATE BIOSYNTHESIS BY FUNGUS LEPTOSPHAERIA MACULANS DURING INTERACTION WITH THE HOST PLANT

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Key words: *Brassica napus*; Filamentous fungi; *Leptosphaeria maculans*; Salicylic acid; transcriptomics

Leptosphaeria maculans is one of the most destructive pathogens of economically important crop oilseed rape, *Brassica napus*. *L. maculans* is spread by ascospores, causing lesions in leaves at early stages and ending up with stem canker. Upon colonization *L. maculans* produces a range of secondary metabolites to manipulate and evade host plant immunity. Plants have a repertoire of defense mechanisms against fungal invasion, a particular role among which plays salicylic acid (SA). Infection by *L. maculans* triggers SA biosynthesis in plant tissues, which is indispensable for establishing immunity, and regulating programmed cell death. Brassicaceae have 2 major pathways of SA synthesis known as PAL and ICS. PAL pathway is

named after enzyme Phenylalanine Ammonia-Lyase which converts phenylalanine to trans-cinnamic acid then to benzoic acid, whose hydroxylation results in SA. ICS (isochorismate synthase) pathway starts in the chloroplasts by converting chorismate derived from shikimate pathway into isochorismate via ICS (isochorismate synthase), which is then transported into cytosol. Some of *L. maculans* effector proteins are known to have reductive effect on SA synthesis, while the fungus was able to accumulate and secrete SA in vitro. We hypothesized that *Lm* may also possess a functional biosynthetic machinery and use it during plant colonization. Through ortholog profiling and transcriptomic analysis of a time-course experiment, we have identified 5 *L. maculans* genes potentially involved in pathogenesis and SA-synthesis, 2 genes being orthologous to plant genes encoding key proteins involved in ICS and PAL pathways, the rest being orthologous to genes responsible for bacterial SA synthesis. The in-silico data are now being validated by qRT-qPCR with candidate-gene-based primers coupled with measurement of SA levels across compatible-incompatible interactions and in vitro fungal cultivation. Discovering the mechanism and role of SA biosynthesis in plant pathogenic fungi will open a new chapter in our understanding of plant-fungi interaction and plant protection.

Acknowledgement

This work is supported by project TowArds Next GENeration Crops, reg. no. CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes Amos Comenius.

THE EVALUATION OF SILICON APPLICATION ON THE GROWTH OF TWO AMARANTH CULTIVARS UNDER SALT STRESS

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Key words: amaranth, morphological parameters, salinity, silicon

Plant growth in saline soils is adversely affected in multiple ways, including reduced biomass, stunted height and decreased yield due to salt stress. The global expansion of salt-affected areas poses a serious threat to future food security. Therefore, there is growing interest among researchers and farmers in identifying crop species that not only tolerate saline conditions but also respond positively to beneficial substances applied as fertilisers. Grain amaranth is recognised for its remarkable adaptability and ability to thrive in degraded soils. In Slovakia, several amaranth cultivars have been bred for inclusion in the food fund of Central Europe. However, their tolerance to salinity and the potential ameliorative effects of silicon (Si) have not yet been thoroughly investigated.

This study aimed to evaluate the tolerance levels to salinity and Si in the first Slovak amaranth cultivar and its parental line. We assessed the morphological responses that occurred after the application of sodium chloride (NaCl) and examined the effect of Si applied as a foliar spray during the 7th and 8th weeks of growth. Morphological traits measured included root and shoot length, as well as fresh and dry biomass.

The most pronounced impact of NaCl was observed on root parameters. Salinity substantially increased the fresh and dry weight of roots in both cultivars and enhanced root length in the parental cultivar. Although the Si application did not influence root biomass, the water content in the roots of salt-stressed amaranth was considerably higher following Si supplementation. In the Slovak cultivar, foliar Si application led to substantial improvements in shoot length, biomass, and water content. In contrast, the parental cultivar showed improvements in these parameters only under salinity stress, except for shoot dry weight, which increased notably after Si application on plants grown in non-saline conditions.

Acknowledgement

This work was supported by the Scientific Grant Agency VEGA, grant number 2/0013/22, and COST Action CA22144.

DIFFERENTIAL RESPONSE OF MAIZE ROOT CATEGORIES TO ARSENIC TOXICITY: THE ROLE OF SILICON IN LIGNIFICATION AND ANTIOXIDANT ACTIVITY

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Key words: arsenic, lignification, oxidation stress, root categories, silicon

Arsenic (As) is one of the most toxic environmental contaminants, severely affecting plant growth and metabolism through the induction of oxidative stress and disruption of essential physiological processes. This study investigates the responses of three root categories (main, adventitious, and nodal) in two maize hybrids (Tweeter and Luciana) to two levels of arsenic exposure (75 μ M and 150 μ M) and the mitigating effect of silicon (Si) application, with a specific focus on lignification and antioxidant enzyme activity. Three-way ANOVA revealed that treatment was the most significant factor affecting the measured parameters, with varying responses across hybrids and root types. Arsenic exposure notably reduced root length and guaiacol peroxidase (G-POX) activity, particularly in adventitious and nodal roots, while tyrosine ammonia-lyase (TAL) activity peaked under high As concentrations. The addition of Si partially alleviated these negative effects, especially in nodal roots of Tweeter. Lignin and soluble phenolics (GAE) content were most affected in adventitious roots, with strong correlations between G-POX, PAL (phenylalanine ammonia-lyase), and GAE, indicating activation of the phenylpropanoid pathway as a defense mechanism. Principal component analysis (PCA) distinctly separated As-treated roots from control and Si-treated samples, confirming a coordinated antioxidant and lignification response under As stress.

The results demonstrate root-type-specific responses to As toxicity and suggest a selective, though not universal, protective effect of Si in mitigating arsenic-induced stress. These findings underscore the importance of considering root system complexity and genotype variability in phytotoxicity and stress amelioration studies.

Acknowledgement

The recent work was financially supported by the Slovak Research and Development Agency under contract Nr. APVV-17-0164, and VEGA1/0472/22.

INVESTIGATING THE IMPACT OF HEAT STRESS ON FEMALE GAMETOPHYTE DEVELOPMENT AND SYNERGID CELL FUNCTION IN *ARABIDOPSIS THALIANA*

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Key words: female gametophyte development, heat stress, kinases, ROS, synergid cell

Successful fertilization of the ovule is, among others, dependent on the synergid cells, which are releasing chemoattractants to guide the pollen tube to the ovule. In my PhD project, I will investigate the premature degeneration of the synergid cells during heat stress on a genetic and molecular level. I hypothesize that this premature degeneration is due to premature activation of the programmed cell death pathway involving a protein phosphorylation cascade and resulting in ROS-production. Based on this, I am assessing the involvement and function of several serine/threonine kinases and doing this by phenotyping the ovule structure and the synergid cell function in the corresponding mutants under heat stress condition. Subsequently, I will find the targets of the kinases under investigation by conducting phosphoproteomic assays. In addition, I am

also monitoring the impact of heat stress to the female gametophyte (FG) development using various biosensors and FG markers. With my PhD project, I aim to identify involved pathways ensuring synergid cell fitness and function during heat stress as well as to define the main impact of heat stress on the FG development.

Acknowledgement

Plant Sciences Core Facility of CEITEC Masaryk University and the core facility CELLIM supported by the Czech-Biolmaging large RI project (LM2023050 funded by MEYS CR) is gratefully acknowledged for the obtaining of the scientific data presented this presentation. This research is supported by OP JAK TowArds Next GENeration Crops (TANGENC) reg. no. CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes Amos Comenius.

EFFECTS OF FERMENTED NETTLE EXTRACTS ON ZEA MAYS L. PLANTS DURING DROUGHT STRESS

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Key words: antioxidant properties, biostimulant, glycosidases, proteases

Biostimulants are substances of natural origin that, when applied to plants, increase their growth, nutrient uptake, yield and quality of fruits while also influencing their defense response to stress. Fermented nettles have long been used in traditional horticulture to increase growth, resistance and yield of garden crops. They present a rich source of nutrients, essential microelements as well as a variety of beneficial microorganisms. However, the exact mechanism of action of this potential biostimulant is still unknown. The fermented nettles (FN) used in this study were firstly characterized in terms of various biochemical parameters: contents of proteins and saccharides and the activity of hydrolytic enzymes related to their degradation, as well as total phenolic compounds and antioxidant power. In the next step, *Zea mays* L. (DKC 3969) plants repeatedly treated with FN, in the form of soil drench, were exposed to drought stress. The FN-treated plants fared better in mitigating the detrimental effects of drought stress than the untreated controls. This was reflected in the contents of leaf pigments, the activity of photosynthetic enzymes and also in the activity of glycosidases. The activities of the antioxidant enzymes (peroxidases, superoxide dismutase, ascorbate peroxidase, and glutathione reductase) which strongly increased in plants exposed to stress, were lowered in FN-treated plants. Together these results indicate that FN-treatment helps to alleviate the negative effects of drought stress by inducing changes in plant antioxidant system, nutrient management and photosynthetic system.

Acknowledgement:

This research was funded by Technology Agency of the Czech Republic, SQ01020132 and by Charles University, Cooperation Program, research area Biochemistry, SVV 260820/2025.

THE SURVEY AND MAPPING OF PLANTS FOR ECOTOURISM IN THE SURROUNDING AREAS OF THAM LUANG-KHUN NAM NANG NON NATIONAL PARK (PREPARATORY), CHIANG RAI PROVINCE

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Key words: forest surveys; GIS; national parks; NDVI; satellite images

This research focuses on surveying and mapping plant species for Ecotourism in the surrounding areas of Tham Luang-Khun Nam Nang Non National Park (Preparatory), Chiang Rai Province, using Geographic Information System (GIS) technology and the Normalized Difference Vegetation Index (NDVI) analysis from Landsat 8 satellite images. The study evaluates biodiversity and vegetation density in the area, which includes mixed deciduous and montane forests that are rich in natural resources and biodiversity. Field surveys were conducted along four nature trails, using GPS data to record the geographic coordinates of each plant species. The collected data was analyzed and mapped with ArcGIS software for accuracy. The plant species were verified through academic databases and field validation. The NDVI analysis highlighted vegetation health and greenness levels. NDVI values, calculated using wavelengths of 0.63–0.70 μm (red) and 0.7–1.1 μm (near-infrared), range from -1 to 1. Results showed that NDVI values in 2024 (densely vegetated areas 55%) were higher than in 2015 (densely vegetated areas 42%), indicating improved vegetation density. This study developed a vegetation map and a Normalized Difference Vegetation Index (NDVI) map using the ArcGIS program, which is highly significant for long-term natural resource conservation planning. The findings can support the development of eco-trails and sustainable forest conservation plans. Moreover, the data can serve as a guideline for restoring degraded forest areas affected by climate change and human activities in the future.

Acknowledgement

We express our profound gratitude to the personnel of Tham Luang - Khun Nam Nang Non Forest Park for their invaluable facilitation, provision of expert knowledge, geospatial data, and on-site assistance crucial to our research, survey, and mapping endeavors.

COMPARATIVE STUDY OF GAS CHROMATOGRAPHY AND PHOTOACOUSTIC DETECTION FOR MEASURING ENDOGENOUS ETHYLENE IN PLANTS

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Key words: ethylene, gas chromatography, photoacoustic detection

We developed and compared gas chromatography (GC-FID) and photoacoustic detection (ETD-300) for measurement of ethylene production in plants. Both of these methods have been used in the past to measure ethylene production in plants, but a detailed comparison of these approaches and an attempt to merge them into a single pair have not yet been published. Our aim was to develop an approach, combining the advantages of both methods. While the use of GC has been proven to be very effective and requiring small amounts of sample gas, photoacoustic detection provides more sensitive measurements. Our approach involves measuring plant fresh mass which is giving us a possibility to determine the endogenous levels per unit fresh plant weight. This allows us to recognize whether the change in ethylene production was due to an increase in plant fresh weight and/or whether the treatment actually affects ethylene production per se. Our approach is also unique in that unlike typical methods, where plants are grown on treated media, we grow them on untreated media and the plant treatment is applied at a precise time period. This allows us to eliminate the effect of the treatment on germination and more accurately simulate the effect of tested compounds under field conditions. Another advantage is that the subsequent use of the combined approach on the same sample allows us to recognize machine errors that would otherwise be attributed to the biological variability. We are also able to save the samples for further hormonal measurements by mass spectrometry.

The method has been successfully tested for use with Arabidopsis plants treated with compounds known from the literature to affect ethylene production. Although the machines do not provide numerically identical values, the observed trends are identical and the values are in the same order of magnitude.

Acknowledgement

The work was supported from European Regional Development Fund-Project "SMART Plant Biotechnology for Sustainable Agriculture" (No. CZ.02.01.01/00/23_020/0008497).

INTERACTION BETWEEN DEK1 CALPAIN AND AUXIN IN PLANT DEVELOPMENT

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Key words: auxin, DEK1, *Physcomitrium patens*, plant development

Defective kernel 1 (DEK1) is the only calpain identified in land plants (Embryophyta) and plays a critical role in determining proper plant growth architecture. Despite its importance, the activation mechanism of DEK1 and its molecular targets remain largely unknown. Auxin, a plant hormone, regulates nearly all aspects of growth and development. Previous studies suggest that DEK1-regulated developmental processes are strongly influenced by auxins. In this study, we employed auxin signalling reporter lines for targeted genetic modifications of DEK1 in the moss *Physcomitrium patens*. Using the moss-specific ratiometric reporter PpR2D2, we sensed auxin gradients in the tissues of PpDEK1 mutant lines. Our results show that auxin sensing is significantly reduced in the buds of PpDEK1 deletion mutant line compared to the wild-type. Furthermore, we observed differential gene expression related to auxin sensing among the studied lines. These findings suggest that PpDEK1 is important for maintaining proper auxin sensing during the transition from the filamentous to the 3D growth in *P. patens*. Current experiments aim to analyze differences in auxin transport and biosynthesis among the studied *P. patens* lines.

Acknowledgement

This work was supported by VEGA 1/0352/21 and APVV-21-0227 grants.

TOWARDS BIOCONTROL OF BACTERIAL DISEASES: NEW PSEUDOMONAS PHAGE ISOLATION, CHARACTERIZATION, AND GENERATION OF A LIBRARY OF TN5 MUTANT P. SYRINGAE PV TOMATO

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Key words: bacteriophages, *Pseudomonas syringae*, characterization, mutant library

In the context of reducing the use of hazardous agrochemicals and mitigating bacterial resistance to them, bacteriophages (phages) appear to be promising biocontrol agents for crop protection against bacterioses.

We aim to develop a functional phage-based solution to combat bacterioses of tomato. To do so, we have isolated a set of novel phages from the environment, and are now characterizing their properties, mechanisms of interaction with the host, and efficiency against *Pseudomonas syringae* pv. *tomato* (*Pst*) in *Arabidopsis thaliana* model plant. Two new phages have been isolated from pepper fruits (Pap5 and Pap7), which appeared to be stable at a range of environmentally relevant pH and temperatures, and efficient against an array of *Pseudomonas* bacteria *in vitro* and *in planta*. We have developed a method of phage formulation and spray application which enhanced phages persistence on leaves and ensured efficiency when applied before the bacterial inoculation. We are currently investigating the bacterial receptors which are targeted by these phages using Tn5 transposon mutagenesis. A library of mutated *Pst* strains has been prepared and will be exposed to the phages, followed by individual sequencing of the resistant colonies. We aim at identifying the *Pst* genes involved in phage adsorption, which will help us understanding the mechanisms of phage resistance and predict wider host range through bioinformatics comparison. This prediction would help us to ensure treatments efficiency by developing phage mixtures that cover a wide enough spectrum of bacterial receptors, avoiding emergence phage resistance through single mutation.

Acknowledgement

This work is supported by Technological Agency of Czech Republic (TAČR, TQ03000088). IEB Imaging Facility is supported by MEYS, grant LM2023050 „Czech-BioImaging“. The project of generation of the library of *P. syringae* mutants was supported by STSM grant of the COST Action CA22158 MiCropBiomes.

DO PLANTS USE THE ACTIN CYTOSKELETON IN DNA REPAIR?

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Key words: ARP2/3 complex, arpc5, DNA repair, DSB, HR

DNA double-strand breaks (DSBs) pose a significant threat to the cell, as they can lead to chromosome disintegration and cell death if they are left unrepaired. For this reason, a few distinct repair pathways have evolved, including homology-directed repair pathways such as single-strand annealing (SSA) and homologous recombination (HR). It appears that this complex repair pathway must cooperate with the cytoskeleton, as well as other cellular systems. In animals, it has been shown that the cytoskeletal complex ARP2/3 (which enables de novo nucleation and branching of actin filaments) plays a critical role in HR. However, there is no such evidence of the cytoskeletal role in DNA repair in plants. Therefore, to investigate the possible role of ARP2/3 on DNA repair, we have crossed *Arabidopsis* lines mutated in subunits of the ARP2/3 complex with the fluorescent marker line for HR, RAD54-YFP, allowing us to track and compare the repair pathway in real time in individual cells. To further assess how the loss of ARP2/3 affects the choice of DNA repair pathway over time at the whole-plant level, we performed a homologous recombination assay in ARP2/3 mutant backgrounds. We found a significant increase in the number of DSBs that are being repaired by HR in individual cells, and also an increase in total repaired lesions by both SSA and HR pathways in the arpc5 background, one of the ARP2/3 complex subunits. These results indicate the role of ARP2/3 in the maintenance of genome stability or the DNA repair itself; however, further research is still needed.

SPRAY-INDUCED GENE SILENCING AS A STRATEGY TO PROTECT *PAPAVER SOMNIFERUM* AGAINST *BOTRYTIS CINEREA* AND *APHIS FABAE*

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Key words: *Papaver somniferum*, *Botrytis cinerea*, Aphids, RNAi, SIGS

The Czech Republic is a global leader in the cultivation of *Papaver somniferum* for food industry, where it holds cultural and culinary significance. However, like other crops, *P. somniferum* is susceptible to various pathogens and pests that negatively impact yield. Among the most common are the fungal pathogen *Botrytis cinerea*, which causes grey mold on foliage, and aphid species such as the poppy aphid (*Aphis fabae*) and the green peach aphid (*Myzus persicae*). Intensive and widespread use of fungicides and pesticides could pose significant risks to the environment and potentially human health- EU has in its „Farm to Fork“ strategy the aim to decrease the usage of chemicals up to 50 %. It brings demand for new more specific and environmentally friendly crop protection strategies. Such strategy represents usage of RNA interference in the approach called Spray-Induced Gene Silencing (SIGS). In SIGS alternatively, synthetic double strand RNAs (dsRNA) are applied topically to the plant surface with the aim to be uptaken by pathogen or pest and silence their essential gene(s) . Which should lead to significant decrease of their viability.

In our work we established poppy pathosystems with the above-mentioned pathogen and pests under control conditions and we designed and produced dsRNA(s) specifically targeting them. We tested the effect of our dsRNAs on our pathosystems.

We believe that RNAi approach represent a promising, species-specific, and eco-conscious tool for protecting *P. somniferum* from biotic stress and once established the pipeline could be used for protecting *P. somniferum* against plethora of pathogens and pests.

Acknowledgement

We would like to thank for financial support from MEYS Inter-Excellence II, Inter-COST project nr. LUC23146.

DETECTION OF PROTEIN S-NITROSATION IN PLANTS: BIOTIN SWITCH VERSUS SNO-RAC METHODS

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Key words: biotin switch; redox modifications, S-nitrosation

Posttranslational modifications of protein cysteines, including oxidation, S-nitrosation and persulfidation, are important mechanisms that regulate protein biological activity and localisation. The most important source of biologically available nitric oxide is apparently S-nitrosoglutathione (GSNO), which is believed to be involved in the control of protein S-nitrosation status. We have tested and optimized the detection method of S-nitrosated protein cysteines known as SNO-RAC (resin-assisted capture of S-nitrosated proteins). We also compared this method to a more commonly used biotin-switch technique (BST). Individual steps of both methods were modified to achieve a higher yield of isolated S-nitrosated proteins. Both methods were experimentally tested using bovine serum albumin as a model protein as well as proteins extracted from tobacco cell culture (*Nicotiana tabacum* cv. Xanthi). The influence of important parameters such as detergent choice, concentrations and volumes of key reagents, the length and the way of incubation of proteins with matrices, was examined. It has been concluded that the BST method shows higher detection sensitivity and easier work with neutravidin matrix, on the other hand, lower price and shorter time of sample processing can be considered as the advantage of SNO-RAC method.



26th–28th August 2025

Plant Biology CS 2025



Abstracts

ORAL LECTURES

BUILDING A PLANT BODY

INVITED
PLENARY
SPEAKER

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Key words: Marchantia, meristem, polarity, stem cells

The multicellular bodies of plants develop from single cells; the multicellular diploid phase of the plant life cycle develops from a polarised zygote, and the multicellular haploid phase develops from a spore without polarity. Each of these cell types divide to form masses of cells – enclosed embryos and free-living sporelings respectively. These cell masses form meristems, generative centres from which the mature body of the plant develops.

The *Marchantia polymorpha* spore is produced by meiosis and lacks any markers of polarity. Upon germination, the spore polarizes de novo. This polarity orients the first cell division, which is asymmetric and produces a larger cell on apical side and a smaller cell on the basal side. The apical cell functions as a generative cell and divides while the basal cell terminally differentiates as a rhizoid cell. Preliminary data indicate that light polarises the spore cell and orients the first asymmetric cell division. A stem cell niche develops in cells derived from the apical cell and forms the plant body. Recent data on mechanism that operates during the development of cell polarity and the initiation of the meristem will be presented.

Acknowledgement

This work was carried out by team of researchers in our lab. It was funded by the European Research Council and the Austria Academy of Sciences.

HOW NOVEL CELL WALL-DIRECTED PROBES HELPED DISCOVER SOME UNEXPECTED BIOLOGICAL PHENOMENA

INVITED
PLENARY
SPEAKER

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Key words: aptamer, cell wall, imaging, probing, pectin, root development

Detection of cell wall components *in situ* is a crucial experimental step for studying the structure and function of plant cell walls. Despite the already existing large repertoire of anti-glycan monoclonal antibodies, there are still inconvenient gaps in polysaccharide epitope coverage and methodological limitations, including immunoglobulins' large molecular weight, hampering direct *in vivo* analysis and real-time imaging. Over the last 10 years, we successfully introduced novel molecular tools into our research, such as homogalacturonan-recognising oligosaccharide-based probes, cellulose-binding DNA

aptamer, click chemistry-enabled monosaccharides and quantum dots for cell wall morphometric analysis. Experimental advances helped us to discover some interesting phenomena, including shank-localised growth of Arabidopsis root hairs or non-degradative separation of root border cells. I will present some successful examples of elucidating these phenomena, focusing on pectins and root development. Finally, I will present our current efforts, including the development of *in vitro* selected and in silico designed cell wall probes. We expect that these new tools will enable us, for the first time, to visualise 3-dimensional cell wall structures, such as covalent and non-covalent interlinks between macromolecules, and hence should contribute to a better understanding of cell wall architecture and dynamics in different biological contexts.

Acknowledgement

This research is funded by the program IMPULZ (grant IM-2021-23) of the Slovak Academy of Sciences.

FUNCTIONAL DIVERSIFICATION OF EXOCYST COMPLEX SUBUNITS, PLANT TERRESTRIALIZATION AND POLYSPORANGIATE ORIGIN OF LAND PLANTS

**INVITED
PLENARY
SPEAKER**

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Key words: alternation of generations, defense, exocyst complex, EXO70, plant terrestrialization

The vesicle tethering complex exocyst assists in localized delivery of exocytosis vesicles to specific PM domains. This function is especially significant within the context of sessile land plants cells engaged within the cell walls apoplastic network. Land plant exocyst subunits evolutionary multiplication correlates closely with the plants terrestrialization. This is esp. apparent in the case of EXO70 family forming three distinct subfamilies at the dawn of plant conquering dry continents from fresh water bodies. Even closely related EXO70s might be functionally very specific and omic analyses indicate that most cell types express and use several different EXO70 isoforms. EXO70 isoforms are implied also in unconventional secretory processes related to autophagy, secondary metabolites transport, cell growth regulation, signalling, secondary cell wall biogenesis (including callose and silica deposition) and defence. Engagement of EXO70.2 class of EXO70s in biotic interactions and defence correlates well with enormous production and conservation of new EXO70 variants within this class. But what ecological conditions triggered transition of streptophyte ancestors of land plants from water to land and what was the developmental status of common ancestor of bryophytes and tracheophytes? We will discuss these unresolved questions also within context of plant exocyst evolution.

Acknowledgement

Our work in this area is supported by the GACR/CSF projects 23-05564S; 24-12829S, 25-18138S and TowArds Next GENeration Crops/TANGENC, reg. no. CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes Amos Comenius.

Session 1:

**Cell biology, cell cycle
and cell development**

GENETIC ANALYSES OF PLANT CALPAIN DEK1 IN *PHYSCOMITRIUM PATENS* UNCOVER MULTIPLE LAYERS OF ITS ESSENTIAL ROLE DURING GROWTH AND DEVELOPMENT

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Key words: calpain protease, cell fate, plant development, proximity labeling

Plants contain a single calpain protease - the DEFECTIVE KERNEL 1 (DEK1). DEK1 is a 250 kDa multi-domain membrane protein with essential functions throughout plant development. In cereals for example, DEK1 controls nutrient-rich aleurone cells differentiation in the endosperm. Its function is essential for epidermal cell fate maintenance throughout plant ontogenesis with impact on shoot apical meristem, vegetative and reproductive organs development. Despite its essential functions, the molecular mechanism of DEK1 function is still enigmatic. The main unanswered questions include DEK1's spatio-temporal regulation, and identity of its calpain-domain substrates. In our lab we use reverse genetics in the model plant *Physcomitrium patens* to dissect the function of DEK1 at cellular and molecular levels. In my presentation, I will share our current efforts to understand how specific DEK1 domains contribute to the protein function in different tissues. We also performed *DEK1* mutagenesis in diverse *P. patens* reporter strains, which provided hints to its putative calpain substrates. To further investigate the DEK1 *in vivo* interactome, we generated transgenic lines in *P. patens* WT and *Ddek1* background for so called *proximity labeling*. Initial results from this experiment will be also discussed.

Acknowledgement

This work was supported by The Slovak Research and Development Agency grants APVV-17-0570 and APVV-21-0227.

THE ROLE OF CLASS I FORMINS AT PLASMODESMATA

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Key words: actin, formin, plasmodesmata, symplastic movement

Symplastic transport among plant cells is an important cellular process where molecules move to neighboring cells through plasmodesmata, which can regulate this transport by controlling the size exclusion limit (SEL). Developmental factors and environmental cues can influence the SEL of the plasmodesmata. Class I formins are a family of membrane-anchored actin nucleators that have multiple roles in the growth and development of roots. The Arabidopsis formins AtFH1 and AtFH2 have been found to localize to the plasmodesmata in the root meristematic zone, as well as in above-ground tissues, and influence the symplastic transport in the later.

In this study, we investigate the roles of formin single mutant *atfh1* and *atfh2* and double mutants *atfh1atfh2* in root development. Our first objective was to establish a link between formins and callose deposition at the cell plate, which we assessed through staining and microscopy. Subsequently, using the light-switchable fluorescent protein DRONPA for live-cell tracking and imaging, we demonstrated the significance of formins in regulating symplastic transport. In the final part of our study, we explored the connection between formins and lateral root formation. Collectively, our results suggest that formins may modulate the SEL of plasmodesmata, thereby affecting intercellular trafficking and ultimately leading to physiological changes in plants. Additionally, we characterized AtFH2 in roots by examining its subcellular localization and associated traits. To further elucidate the function of AtFH2, we employed several pharmacological treatments including the formin inhibitor SMIFH2, the actin polymerization inhibitor Latrunculin B, as well as wortmannin and concanamycin to probe the nature of vesicles carrying the AtFH2 signal and to determine the role of AtFH2 in this process. Overall, our work provides a comprehensive investigation into the cellular roles of class I formins and their contributions to plant development and intercellular communication.

Acknowledgement

This work has been supported by the Czech Science Foundation grant 22-33471S.

KEEPING COOL UNDER PRESSURE: HOW EIF3 SAFEGUARDS POLLEN FERTILITY AND THERMOTOLERANCE

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Key words: heat stress, plant reproduction, pollen development, thermotolerance, translation initiation

Pollen germination and pollen tube growth are highly sensitive to elevated temperatures, requiring precise translational control to maintain cellular integrity and reproductive success. The eukaryotic translation initiation factor 3 (eIF3) plays a crucial role in orchestrating mRNA translation dynamics, impacting both pollen tube growth under normal conditions and thermotolerance during heat stress (HS). We demonstrate that disruption of specific eIF3 subunits leads to impaired pollen tube growth and structural integrity, affecting fertilization efficiency. However, under heat stress, translational adaptation mechanisms involving eIF3 subunits contribute to enhanced pollen tube thermotolerance by regulating heat shock protein (HSP) expression. We further reveal that eIF3 interacts with specific mRNA elements, modulating their translation through a balance of repression and activation. Structural and functional analyses highlight the importance of conserved eIF3 domains and post-translational modifications in maintaining translational equilibrium essential for pollen tube membrane integrity and sustained growth. Collectively, our findings uncover a pivotal role of eIF3-mediated translational regulation in ensuring pollen fertility under both optimal and stress conditions.

Acknowledgement

The authors gratefully acknowledge the financial support from Czech Science Foundation, grants No. 23-07000S and 24-10653S, from European Cooperation in Science and Technology COST Action RECROP (CA22157), and from the European Regional Development Fund (ERDF) Programme Johannes Amos Comenius project TowArds Next GENeration Crops (TANGENC) (reg. no. CZ .02.01.01/00/22_008/0004581).

ARO-DEPENDENT CHANNELLOSOMES ARE ESSENTIAL FOR VARIETY OF RAPID CELLULAR RESPONSES, FROM AUXIN TO ABSCISIC ACID, ACROSS ALL LAND PLANTS

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Key words: CNGC, Calcium, alkalization, channel

Plant cells detect a variety of stimuli through intracellular calcium (Ca²⁺) signaling. Cyclic nucleotide-gated channels (CNGCs) constitute a key family of plant Ca²⁺ channels, with 20 homologs identified in Arabidopsis. These tetrameric plasma membrane proteins function downstream of diverse signals, including phytohormones, extracellular damage, cell wall integrity, and temperature changes. We identify a unique class of plant-specific proteins, Armadillo Repeat Only (ARO), as critical regulators of almost all plant CNGCs. Loss of functional AROs in sporophytes induces phenotypes that closely resemble CNGC dysfunction, such as impaired root gravitropism, abnormal root hair growth and morphology, stomatal movement defects, and altered responses to extracellular ATP and auxin. *aro2/3/4* mutants fully resist the toxic effects associated with CNGC overexpression. We demonstrate that AROs colocalize with and physically interact with multiple CNGCs, influencing CNGC-dependent ion currents in *Xenopus* oocytes. Structural modeling combined with site-directed mutagenesis suggests that AROs form tetramers around the CNGC channel, interacting via the channel's IQ domain. Collectively, our findings reveal that plant CNGCs do not function in isolation but as part of a larger complex - "channelosome" marking the first such discovery in plants.

Acknowledgement

This project was supported by the Czech Science Foundation grant Nr. 25-16449S and by European Union, Horizon Europe, project MOLIPeC, ID 101087030.

SUSPENSION ESTABLISHMENT AND HEAT SHOCK RESPONSE OF GOLDEN GARDENIA (*GARDENIA SOOTEPENSIS HUTCH.*)

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Key words: *Gardenia sootepensis* Hutch., Plant tissue culture, Cell suspension culture, Heat stress response

Gardenia sootepensis Hutch., known as golden gardenia, is a medicinal evergreen tree native to Southeast Asia with notable antimicrobial, anti-inflammatory, cytotoxic, and antioxidant properties. Despite its pharmacological value, little is known about its cellular responses to abiotic stress, particularly heat stress. This study aimed to develop in vitro culture protocols and investigate heat tolerance in suspension cells derived from leaf explants. Callus induction was successfully achieved using Murashige and Skoog (MS) medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D; 0.5–2.0 mg/L) and kinetin (Kn; 0.1 or 0.2 mg/L), yielding a 100% induction rate. Calli formed with 0.5–1.0 mg/L 2,4-D were friable and olive-green, while 2.0 mg/L produced browning. Suspension cultures established with 0.5–1.0 mg/L 2,4-D and 0.1 mg/L Kn showed enhanced cell proliferation and biomass accumulation. Heat shock experiments revealed that *G. sootepensis* suspension cells tolerated exposure to 55°C (extracellular medium temperature of 46.7 ± 0.10°C) for 5 minutes without visible structural damage. These results demonstrate the species' potential for in vitro propagation and its initial resilience to heat stress. Further research is needed to refine culture conditions, assess long-term stability and genetic fidelity, and deepen understanding of stress response mechanisms.

Acknowledgement

This research was supported by University of Phayao, and Demonstration School, University of Phayao.

Session 2:

**Carnivorous and aquatic plants,
bryophytes and lichens**

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Key words: ex situ, functional traits, liverworts, mosses, survival

The rapid environmental and climate changes cause the distinction and developing novel discipline within life science, namely Conservation Physiology. It modestly includes also to the organisms of non high economic values like bryophytes. The research area includes studies of functional traits of rare and threatened taxa with aim to enhance existence both in *ex situ* and *in situ* conditions and aim to achieve biological entity survival. The approach is rather experimental and it is related to integrative conservation. Bryophytes, being the second biggest group of terrestrial plants play pivotal roles in many ecosystems functions and can serve as a source of novel material for modern applications as nature inspired solutions in everyday life and also in various industrial branches. The high population decrease of many species led to urgent need of active conservation and thus protection measures and activities should be not only empirically but also experimentally scientifically supported. The huge bryophyte *ex situ* and *in vitro* collection (over 360 species from all over the world) from the University of Belgrade (Serbia) mirroring also at PJS University in Košice (Slovakia), is a unique approach to study conservation physiology features of bryophytes in fully controlled conditions and deliver many new and interesting data confirming or opposing to those previously believed, at the same time enabling species self-sustainability in nature, successful reintroduction, translocation or population establishing and strengthening. Apart, many novel knowledge came out during the experimentation on rare and threatened taxa since previously it was not marked in nature due to its seldom nature and small appearance. Here, we present some of the most interesting selected case studies and features on rare and threatened taxa achieved so far through conservation physiology approach to bryophytes.

MOLECULAR CLONING AND EXPRESSION PROFILE OF A UNIQUE PROTEASE FROM CARNIVOROUS SUNDEW

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Key words: *Drosera binata*, gene expression, hydrolytic enzymes, molecular cloning

Carnivorous plants are a diverse group of flowering plants that grow in nutrient-poor and mostly wet habitats. To compensate for limited macroelements, they attract arthropods and capture prey using specialized trap leaves, subsequently triggering the expression of specific genes coding for digestive enzymes. Our understanding of transcriptomic changes upon insect capture and the tissue-specific expression of hydrolytic enzymes is still fragmentary and needs deeper investigation. Furthermore, proteins discovered in the trap secretions are a valuable source of hydrolases for various biotechnological applications. Therefore, the aim of this study was to acquire the complete gene sequence of a putative cysteine protease, previously identified through proteomic profiling of induced trap leaves from carnivorous sundew, and evaluate its transcription dynamics in different plant organs. The forked-leaved sundew, *Drosera binata*, was aseptically cultivated on supplemented ¼ MS medium. Nucleic acids were extracted from plants using an optimized CTAB protocol. We performed the genome walking method to acquire the full-length hydrolase gene. The open reading frame of 1168 nucleotides was predicted to encode a protein of 345 amino acids, and comparison of the genomic and cDNA sequences revealed the presence of one intron. RNA from the root, flower, and sequential developmental stages of trap leaves were subjected to probe-based quantitative real-time PCR with gene-specific primers to elucidate the transcriptional



profile of the selected *D. binata* protease. The obtained data were normalized to the expression level of the endogenous reference gene and analyzed using the $2^{-\Delta\Delta Ct}$ method. In conclusion, we isolated a full-length protease-coding gene sequence involved in prey digestion that had not been previously described in sundew, providing a promising candidate for further recombinant protein expression and characterization.

Acknowledgement

The authors would like to thank Anna Fábelová for in vitro plant care and technical assistance. This work was supported by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under project 09I03-03-V04-00573 and project APVV-23-0448.

THE DIVERSITY OF DIGESTIVE SYSTEMS IN CARNIVOROUS PLANTS

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Key words: carnivorous plant, co-option, digestive enzymes, evolution, jasmonic acid

Carnivorous plants have evolved at least 11 times independently in sunny, wet, and nutrient poor habitats and are thus an example of convergent evolution. For carnivorous purpose, they modified the photosynthetic leaves into functional traps which produce digestive enzymes. Despite their independent origin, carnivorous plants surprisingly co-opted similar digestive enzymes from proteins involved in plant defence mechanisms. The plant phytohormone jasmonic acid, involved in regulation of plant defence, was also co-opted for regulation of expression of digestive enzymes in some genera of carnivorous plants. However, recent studies have shown that this happened only once in the oldest Caryophyllales order, and other lineages of carnivorous plants regulate enzyme activity differently. In this lecture, I will show you how different genera of carnivorous plants regulate digestive enzyme activity as well as molecular mechanism how such diversity in digestion may have evolved.

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A close-up photograph of a green, spiny plant stem, likely a poppy. The stem is covered in numerous sharp, white thorns. A dense cluster of dark, oval-shaped aphids is attached to the stem, feeding on the plant's sap. The background is a blurred field of similar plants with green leaves and stems.

Session 3:

Plant microbiome, plant-organisms interactions

THE IMPORTANCE OF MICROORGANISMS IN PLANT ADAPTATION TO THE ENVIRONMENT

INVITED
KEY NOTE
TALK

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Key words: *Arabidopsis*, holobiont, microbiome, microbiota, toxic metals

Natural selection, a core driver of evolution, functions across multiple levels—from individual genes to entire ecosystems. Since Lynn Margulis introduced the concept of the “holobiont” to the life sciences in 1990, it has become increasingly recognized that organisms must be viewed not in isolation, but as integrated systems composed of a host and its associated microorganisms. This collective entity, known as the holobiont, along with the combined genetic content of all its members (the hologenome), forms an evolutionary unit influenced by natural selection. The holobiont’s adaptation to its environment and overall fitness arise from complex interactions between the host and its symbiotic microbes, as well as among the microbes themselves. Microorganisms, due to their rapid adaptability, play a crucial role in enabling the holobiont to detect and respond to environmental changes. The modern understanding of the role of microorganisms in plant adaptation suggests that they are essential for optimizing plant function in the environment.

Acknowledgement

This study was supported by National Science Center, Opus 17 Project, 2019/33/B/NZ9/01372 and Opus 25 2023/49/B/NZ9/01904.

DOES FUNGAL INFECTION INCREASE THE PALATABILITY OF OILSEED RAPE TO INSECTS?

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Key words: *Brassica napus*, *Leptosphaeria maculans*, *Plutella xylostella*, tripartite interaction

In natural conditions, plants have to interact with the other inhabitants of their environment, forming both mutualistic and parasitic relationships. As well as beneficial microorganisms, plants combat a wide range of phytopathogens and insect pests. Plants respond to biotic stress by activating distinct defense mechanisms. However, little is known about how plants cope with multiple stresses. This study aims to investigate the combined effects of fungal infection caused by *Leptosphaeria maculans* and arthropod infestation by the diamondback moth (*Plutella xylostella*) in oilseed rape (*Brassica napus*). We hypothesized that infection by the fungal pathogen *L. maculans* could alter the palatability of oilseed rape to *P. xylostella* chewing caterpillars. To determine possible causes of larval choice, feeding preference tests were complemented with analyses of defense gene transcription and levels of glucosinolates (GLSs) and volatile organic compounds (VOCs) in *L. maculans*-inoculated and non-inoculated (control) leaves. True leaves inoculated with *L. maculans* were more palatable than control leaves to caterpillars during the early stage of infection (3 days post inoculation (dpi)), but this preference disappeared in later stages of infection (7 dpi). In parallel, genes involved in the salicylic acid (SA) and ethylene (ET) pathways were found

to be upregulated in *L. maculans*-inoculated leaves at 3 and 7 dpi. *L. maculans* was also found to increase the level of total aliphatic GLSs, specifically glucobrassicinapin, while decreasing the level of glucoiberin at 3 dpi, as well as altering the content of specific VOCs. The group of 55 VOCs that showed the most variability between the treatments was identified. We suggest that the preference of *P. xylostella* for *L. maculans*-inoculated leaves during the early stages of disease development could be due to underlying mechanisms leading to changes in metabolic composition.

Acknowledgement

The work was supported from European Regional Development Fund-Project " SMART Plant Biotechnology for Sustainable Agriculture " (No. CZ.02.01.01/00/23_020/0008497) co-funded by the European Union.

THE ROLE OF GLUCOSINOLATES IN MUTUALISTIC INTERACTION BETWEEN PLANTS AND THEIR ENDOPHYTIC MICROORGANISMS

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Key words: endophyte, glucosinolates, mutualism, yeast

Glucosinolates (GSLs) are defensive compounds prevalent in *Brassicaceae* plants. They protect plants against herbivores and pathogens but their role in beneficial interactions remains unknown. GSLs themselves don't exhibit antiherbivore activity but are activated upon plant tissue damage. The activation is mediated by enzymes which hydrolyze them into active derivatives. GSLs are also important dietary substances for humans due to their anticancer activity. Besides that, they are considered to be one of the main reasons of general incapability of *Brassicaceae* plants to develop functional symbiosis with arbuscular mycorrhiza.

We observed that *Sporobolomyces ruberrimus* activated *Arabidopsis thaliana* growth. The aim of our study was to explain the molecular background of this interaction and to investigate the role of glucosinolates metabolism in beneficial plant-microorganism interactions.

Global gene expression analysis revealed that GSLs catabolism genes were downregulated in wild type plants (WT) inoculated with endophytic yeast. In the ethylene insensitive *etr1-1* mutants, we didn't observe neither accelerated growth upon inoculation, nor downregulation of GSLs metabolism genes. We found that in WT plants, GSLs abundance didn't change after inoculation, while in *etr1-1* it increased. The abundance of isothiocyanates and nitriles which are active GSLs derivatives were also altered upon inoculation. WT plants actively reduced their concentration while in *etr1-1* it remained unchanged or even increased. To verify how GSLs affect the interaction, we observed 4 different GSLs deficient mutants after inoculation and measured yeast abundance in plants. There were no differences in colonisation between WT plants and mutants but fresh weight of plants was increased only in plants producing indolic glucosinolates. This led us to the conclusion that presence of indolic glucosinolates is crucial for development of mutualistic interaction between plants and their endophytes but their decomposition into active derivatives must be inhibited in order to let the fungi survive in plant tissues.

Acknowledgement

The research was funded under grants 2019/33/B/NZ9/01372 and 2023/07/X/ST4/00710 from the National Science Centre in Poland and supported by a grant from the Malopolska Centre of Biotechnology under the Strategic Programme Excellence Initiative at Jagiellonian University.

THE ROLE OF ARBUSCULAR MYCORRHIZAL FUNGI IN REGENERATIVE AGRICULTURE UNDER THE CONDITIONS OF THE CZECH REPUBLIC

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Key words: arbuscular mycorrhiza, regenerative agriculture, soil health, no-till, cover crops

Arbuscular mycorrhizal fungi (AMF) are crucial for sustainable agriculture and soil health. However, their contribution to regenerative farming—particularly under Central European climatic and agronomic conditions—remains insufficiently understood. Regenerative practices such as no-till farming combined with cover cropping offer great potential for long-term soil carbon sequestration, but the specific responses of AMF under these conditions require further investigation.

This study evaluated the impacts of different soil management strategies on AMF colonisation in winter wheat (*Triticum aestivum*) roots at two long-term experimental sites near Bańín, where field trials have been conducted since 2019. A total of 20 pre-selected plots were assessed, comprising treatments of no-till with cover crops and conventional tillage without cover crops, each under mineral or organic fertilization regimes. Five representative root systems per plot were analysed for AMF colonization.

Our results show that AMF colonisation was significantly higher in no-till plots with cover crops. This is likely due to reduced soil disturbance and the continuous propagation of AMF during the off-season, both of which favour AMF persistence and inoculum preservation. In contrast, conventional tillage combined with wind erosion appears to facilitate spore translocation, potentially destabilizing AMF communities.

These findings highlight the importance of reduced tillage and cover cropping in enhancing AMF networks, which could be pivotal for improving soil resilience and advancing sustainable agricultural systems.

Acknowledgement

This research was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic through the AdAgriF project (CZ.02.01.01/00/22_008/0004635).

THE ROLE OF SEED ENDOPHYTES IN PLANT ADAPTATION TO TOXIC METALS

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Key words: *Arabidopsis arenosa*, microbiota, seed, toxic metals

Symbiotic microorganisms play a key role in plant adaptation to the environment. Very little is known about how metal toxicity impacts the seed microbiota. The aim of this study was to evaluate the effect of toxic metals (TMs) present in the soil on the biodiversity endophytic microbiota inhabiting plant seeds and to describe their role in plant adaptation to TM-polluted environment.

The soil and seed microbiota of model plant *Arabidopsis arenosa* from a Zn-Pb heaps and metal-free ruderal sites were compared. Subsequently, the effect of seed endophytes isolated from these distinct populations on plant growth was determined.



The biodiversity of fungi and bacteria inhabiting mine dump substrate was lower than that of the metal free sites. Soil pollution caused changes in the structure of bacterial communities inhabiting the seeds; the biodiversity of endophytic fungi remained unchanged. The tolerance index of fungi and bacteria to toxic metals was comparable. Endophytic bacteria did not promote plant growth in the presence of metals, while the interaction of fungi was varied and depended on the origin. Fungal strains originating from the seeds of non-metalliferous populations increased plant biomass to a greater extent than isolates originating from metal-liferous populations.

The results indicate that changes in the structure of the microbiota, as a response to environmental changes, will not always explain the increased tolerance of plants to environmental pollution. It may be regulated by certain properties of the microbial strains.

Acknowledgements

This work was supported by National Science Center in Poland (OPUS22 grant no 2021/43/B/NZ9/03034

CSEPB council



THE FUNGUS *ACREMONIUM ALTERNATUM* ENHANCES SALT STRESS TOLERANCE BY REGULATING HOST REDOX HOMEOSTASIS AND PHYTOHORMONE SIGNALING

Invited talk
CSEPB
Award

Berková, V.¹, Berka, M.¹, Štěpánková, L.¹, Kováč, J.², Auer, S.³, Menšíková, S.¹, Ďurkovič, J.¹, Kopřiva, S.⁴, Ludwig-Müller, J.³, Brzobohatý, B.¹, Černý, M.¹

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Key words: *A. alternatum*, beneficial microbe, oilseed rape, salt stress

The study employed a comprehensive approach that assessed plant physiology combined with proteomic, metabolomic, and targeted hormone analyses to elucidate the response of oilseed rape plants to the presence of the endophytic fungus during the initial phase of their interaction. The results showed that inoculated plants under salinity stress increased the fresh and dry weight by approximately 13% and 12%, respectively. Molecular analysis further confirmed a significant effect on the plant

proteome. *A. alternatum* modulated ribosomal proteins, increased the amount of photosynthetic proteins, ROS metabolism, or V-ATPase accumulation. The proteomic analyses reveal an increase in the total amount of antioxidant proteins, which correlates with the observed decrease in hydrogen peroxide and superoxide radicals. *A. alternatum* induced changes in phenylpyruvic acid, cis-ferulic acid, xanthine, and histidine. Findings in proteomics collectively suggested an *Acremonium*-mediated rise in abscisic acid signaling or content, which was subsequently confirmed by targeted phytohormone profiling and analysis of *Arabidopsis* reporter line 6×*ABRE::GUS*. In summary, the results indicate that *Acremonium* promotes salt tolerance by orchestrating abscisic acid signaling, priming the plant's antioxidant system, as evidenced by the accumulation of ROS-scavenging metabolites and alterations in ROS metabolism, leading to lowered ROS levels and enhanced photosynthesis. Additionally, it modulates ion sequestration through V-ATPase accumulation.

Acknowledgement

This work was supported by the Czech-German mobility projects 8J23DE004 and 7AMB18DE015, MSTC Danube project n. 8X23011, and the Internal Grant Schemes of Mendel University in Brno. Reg.no. CZ.0 2.2.69/0.0/0.0/19_073/0016670. SK research is funded by the DFG under Germany's Excellence Strategy – EXC 2048/1 – project 39068611.

PRE-HORMONAL NATURE OF AUXIN UNRAVELED IN STREPTOPHYTE ALGAE

Invited talk
CSEPB
Award

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Key words: auxin, green algae, plant evolution

Auxin is a plant hormone that shows a conserved mode of action across extant land plants. To illuminate the evolutionary origins of auxin's hormonal identity, we turned to the closest living land plant relatives, the streptophyte green algae. We found that the growth reaction and metabolic response of four algal species to applied auxin are unlikely to be attributed to a hormonal function. On the genomic level, the PIN-FORMED (PIN) protein family of transmembrane auxin

transporters is the only major mechanism of auxin action conserved between land plants and streptophyte green algae. Testing multiple algal PINs in both heterologous and homologous expression systems, we discovered that auxin transport function is sometimes observed but is not a universally conserved feature. Indeed, some algal PINs have undergone different evolutionary trajectories. This work opens up some important yet often neglected methodological issues, such as the proper use of controls in treatment experiments, the suitability of heterologous expression models and the hidden variability in algal culture behaviour reflecting the yet improper establishment as model organisms. The story recalls an arduous pursuit of a pioneering project on the way to ultimately attain knowledge.

Acknowledgement

This work was supported by the Czech Science Foundation project 20-13587S.

Session 4:

**Plant genetics, genomics
and epigenetics**



UNRAVELLING THE CONTRIBUTION OF POLYCOMB REPRESSION TO METABOLIC AND DEVELOPMENTAL TRANSITIONS IN PLANTS

INVITED
KEY NOTE
TALK

Mozgová Iva^{1,2}, Samo Naseem^{1,2}, Aflaki Fatemeh¹, Höning Mondeková Helena^{1,2}, Krela Rafat¹, Rivière Quentin¹, Bišová Kateřina³, Michael Borg⁴ et al.

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Key words: chromatin, development, metabolism, plant, Polycomb

Plant development depends on precise and selective control of gene expression in time and space. Our lab is interested in Polycomb repression, an evolutionary conserved epigenetic mechanism that selectively represses genes involved in cell identity specification, development and environmental responses.

By transcriptomic and epigenomic profiling of emerging seedlings of *Arabidopsis thaliana* (*Arabidopsis*), we identified developmental and metabolic pathways that need to be repressed or activated in order for seedlings to stably initiate vegetative (photoautotrophic) growth. We identified genes differentially repressed by Polycomb in source and sink tissues, highlighting the involvement of Polycomb in the control of metabolic states. Having identified analogous phenotypes in *Arabidopsis* and the simple land plant model *Physcomitrium patens*, we ask how well Polycomb repression is conserved in plant evolution from unicellular green algae to land plants. Here, I will take the opportunity to introduce the focus of our lab and present our recent results on the contribution of Polycomb repression to the developmental and metabolic switch during seedling establishment in *Arabidopsis* (Samo et al. 2025) and our search for conserved patterns of Polycomb repression in *Arabidopsis*, moss (Höning Mondeková et al., in prep.) and the unicellular green alga *Chlorella sorokiniana* (Petroll, Papareddy, Krela et al., 2025; Krela et al., in prep.).

References

Samo et al. 2025, TPC – in press (bioRxiv doi: 10.1101/2024.10.08.616934)

Petroll, Papareddy, Krela et al. 2025 MBE (doi: 10.1093/molbev/msaf064)

Acknowledgement

The work in our lab has been supported by Lumina quaeruntur (LQ200961901), ERC-CZ (ERC200961901), CSF (25-17578S) and OP-JAK (CZ.02.01.01/00/22_008/0004624).

SEED COAT-SPECIFIC POLYPHENOL OXIDASE EXPRESSION RESULTS IN HILUM PIGMENTATION

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Key words: domestication, legumes, pea, phenolics, tyrosinase

The seed coat represents a physical, biochemical, and chemical barrier preventing the entry of pathogens or the impact of an adverse environment. Wild and crop plant seeds differ in their seed coat properties as a result of the domestication process.



Oxidoreductases were shown to be important components of developmental and domestication processes in pea. Among them, polyphenol oxidase (PPO) was identified to be differentially expressed between wild and cultivated peas. We used a range of genetic, transcriptomic, proteomic, and metabolomic approaches to determine the function of *PPO* gene in pea seed coat. We found that the functional *PPO* allele is found in all wild pea samples, while the majority (80%) of cultivated peas have non-functional alleles. This corresponds to the *PPO* gene and protein expression as well as the enzymatic activity upregulation in wild peas. We have shown that the functionality of the *PPO* genes relates to the oxidation and polymerization of phenolic compounds (melanin formation) in the seed coat. We heterologously expressed PPO derived from wild pea seed coat (*PeaPPO*) and analysed its biochemical properties. We have shown the tyrosinase nature of *PeaPPO*. Altogether, the seed coat represents a barrier preventing seeds from pathogen invasion and it seems that the hilum is the weakest part (the “Achilles heel”) of this barrier. We hypothesize that phenolic oxidation in the hilum region provided by PPO and its activity is crucial for seed defence against microbial and fungal pathogens.

Acknowledgement

This study received support from the Czech Science Foundation (24- 10730S) and LUAUS25035 project from MEYS CZ.

THE MAIZE B CHROMOSOME EXERTS AN INFLUENCE ON THE TRANSCRIPTOME THROUGHOUT PLANT DEVELOPMENT

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Key words: B chromosome, gene expression, maize

Maize (*Zea mays* L.) is a key global crop as well as a model organism in biological research, with some individuals possessing supernumerary B chromosomes. Although numerous studies have been published characterizing the maize B chromosome, its gene expression patterns across various plant tissues remain insufficiently understood. We established a comprehensive transcriptomic atlas comparing maize plants with and without the B chromosome across eleven tissues and organs. Our analysis shows that B chromosome-encoded genes are actively transcribed throughout plant development, with the highest expression levels observed in reproductive tissues. Co-expression analysis identified a cluster of 30 genes uniquely expressed in the tassel, suggesting their role in regulating crossover frequency mediated by the B chromosome. Beyond its own transcriptional activity, the presence of the B chromosome also influences the expression of A chromosome-located genes across all tissues studied. Our work has deepened the understanding of the transcriptional activity of the B chromosome and its regulatory effects on the maize genome, while also offering valuable resources for future investigations into its broader biological role.

Acknowledgement

The work was supported by the Czech Science Foundation (grant no. 23-04887S) and the ERDF Programme Johannes Amos Comenius (project TowArds Next GENeration Crops, reg. no. CZ.02.01.01/00/22_008/0004581).

WHY SO MANY? INTERPRETING NATURAL VARIABILITY AND GENE EXPRESSION TO UNRAVEL FUNCTION IN LARGE GENE FAMILIES

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Key words: DUF, formin, GWAS, salt stress, transcriptome

Plant genomes contain many large gene families, often of unknown function. In some cases, paralog multiplication is conserved across deep-branching lineages, suggesting maintenance by selection despite expected functional overlap among paralogs. Genome-wide association studies (GWAS) can provide insight into gene function by identifying natural genomic sequence polymorphisms correlated with phenotypic variation, and additional hints on gene function may be inferred from publicly available gene expression data. We performed a GWAS for *Arabidopsis thaliana* genes responsible for phenotypic variability of the leaf epidermis in 310 accessions derived from natural populations. Some of the included traits exhibited possibly ecologically relevant correlation with climatic or physical characteristics of the accession's location of origin. Several large gene families were overrepresented among the candidates for various traits, namely formins (FH2 proteins), cytochrome P450, receptor-like kinases, Cys/His-rich C1 domain proteins, and several "domain of unknown function" families – DUF674, DUF784, DUF1262, DUF1985 and DUF3741 (Bezvoda et al 2025 doi: 10.1111/pce.15357). Examination of expression patterns in the formin family indicated a specific role of one of them, AtFH5, in salt stress response. This was corroborated by changes in GFP-tagged AtFH5 protein localization upon salt treatment, and by increased NaCl sensitivity of loss-of-function *atfh5* mutants (Kollárová et al 2025 doi: 10.1016/j.stress.2025.100770). Remarkably, while AtFH5 was not found in our GWAS study, a large-scale GWAS of 1131 accessions for associations with habitat climatic and physical parameters (Ferrero-Serrano and Assmann 2019 doi: 10.1038/s41559-018-0754-5) identified an AtFH5 missense mutation correlated with climatic parameters determining water availability, further supporting a role of AtFH5 in response to environmental challenges. Additional similar examples will be discussed.

Acknowledgement

The work was supported from the project TowArds Next GENeration Crops, reg. no. CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes Amos Comenius.

PLANT SYNAPTOTAGMINS

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Key words: gene expression, mutant analysis, plant synaptotagmins, protein localization

Synaptotagmins (SYTs) are a family of proteins in animals that play a crucial role in membrane trafficking as calcium sensors, particularly in the nervous system. These proteins contain a transmembrane domain connected by a linker to two calcium-binding C2 domains. Furthermore, extended synaptotagmins in animals and tricalbins in yeast exhibit a similar structure but possess multiple calcium-binding domains and, in addition, a newly defined domain known as the synaptotagmin-like mitochondrial-lipid-binding protein domain (SMP). The SMP, absent in canonical animal SYTs, has been characterized as a lipid transfer module regulating lipid homeostasis at membrane contact sites. Interestingly, plants also have proteins that share characteristics with traditional SYTs, including two calcium-binding C2 domains and transmembrane region, but they also have an SMP domain present in E-SYTs. Six SYTs have been identified in *Arabidopsis*, with *SYT1* being the most extensively studied member. However, most research has been conducted on seedlings growing *in vitro*. Other SYTs in *Arabidopsis* remain poorly understood. In my presentation, I will share our group's findings demonstrating that the lack of SYT1 function reduces photosynthetic efficiency. Additionally,



I will demonstrate how sodium chloride treatment influences the proteomic profile of wild-type *Arabidopsis* and *syt1* roots. I will further present our recent insights into the general characteristics of *SYT3*, *SYT4*, and *SYT5* genes, which have not been previously characterized. Finally, I will outline our results on the genotyping and phenotyping of various *T-DNA* insertion alleles for *SYTs* and discuss our future research directions in this area, including the rationale for incorporating *Physcomitrium patens* into studies.

Acknowledgement

Research in our laboratory is supported by the Slovak Research and Development Agency (APVV) under Grant number APVV-23-0463.

UNVEILING PLANT TELOMERE DIVERSITY: LESSONS FROM *ALLIUM CEPA* TRB PROTEINS

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Key words: Telomere, *telo*-box, TERT, Telomere repeat binding, TRB

Telomeres, the physical ends of linear eukaryotic chromosomes, are typically composed of tandemly repeated short DNA units (e.g., (TTTAGGG)_n in most plants) that are associated with nucleosomal histones and specific telomere-binding proteins. Telomere-binding proteins were extensively characterized in humans, where they form the shelterin complex, which includes Protection of Telomeres 1 (POT1), Telomere Repeat Factor 1 and 2 (TRF1 and TRF2), along with several other proteins. Although the existence of a shelterin-like structure in plants was initially questioned, similar proteins in *Arabidopsis thaliana*, including AtPOT1a, AtPOT1b, and a family of plant-specific Telomere Repeat Binding (TRB) proteins (AtTRB1-5), have been identified. These proteins are responsible not only for telomere protection and telomerase recruitment but also for regulating gene transcription via their binding to the *telo*-box motifs in gene promoter regions, subsequently recruiting PRC2 or PEAT chromatin remodeling complexes.

Despite the high structural conservation of Myb-like domains, variation in telomeric repeat sequences was observed in certain species across the plant kingdom. This is exemplified by the Asparagales order, in which the canonical plant telomere repeat (TTTAGGG)_n has been replaced with non-canonical sequences – specially, human-type repeats (TTAGGG)_n since the divergence of the *Iris* genus, and subsequently with 12-nt telomere repeats (TTATGGGCTCGG)_n following the divergence of the *Allium* plant genus. To investigate the sustained functionality of telomere proteins during these evolutionary changes, we characterize the *Allium cepa* TRB family (AcTRBs), including their subcellular localization and mutual protein-protein interactions. We demonstrate that AcTRBs retain the ability to bind both the ancestral canonical telomeric repeat as well as non-canonical ones. These findings suggest functional plasticity of TRB proteins in response to telomeric sequence evolution.

Acknowledgement

This work was supported for by the Czech Science Foundation [21-15841S] and by the Ministry of Education, Youth and Sports of the Czech Republic under the project INTER-COST LUC24056.

DOMESTICATION AS CONVERGENT AND PARALLEL EVOLUTION – COMPARATIVE ANALYSIS OF CHICKPEA, LENTIL, PEA AND COMMON BEANS FOR TWO KEY DOMESTICATION TRAITS – POD DEHISCENCE AND SEED DORMANCY

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Key words: convergent evolution, domestication, legumes, pod dehiscence, seed dormancy

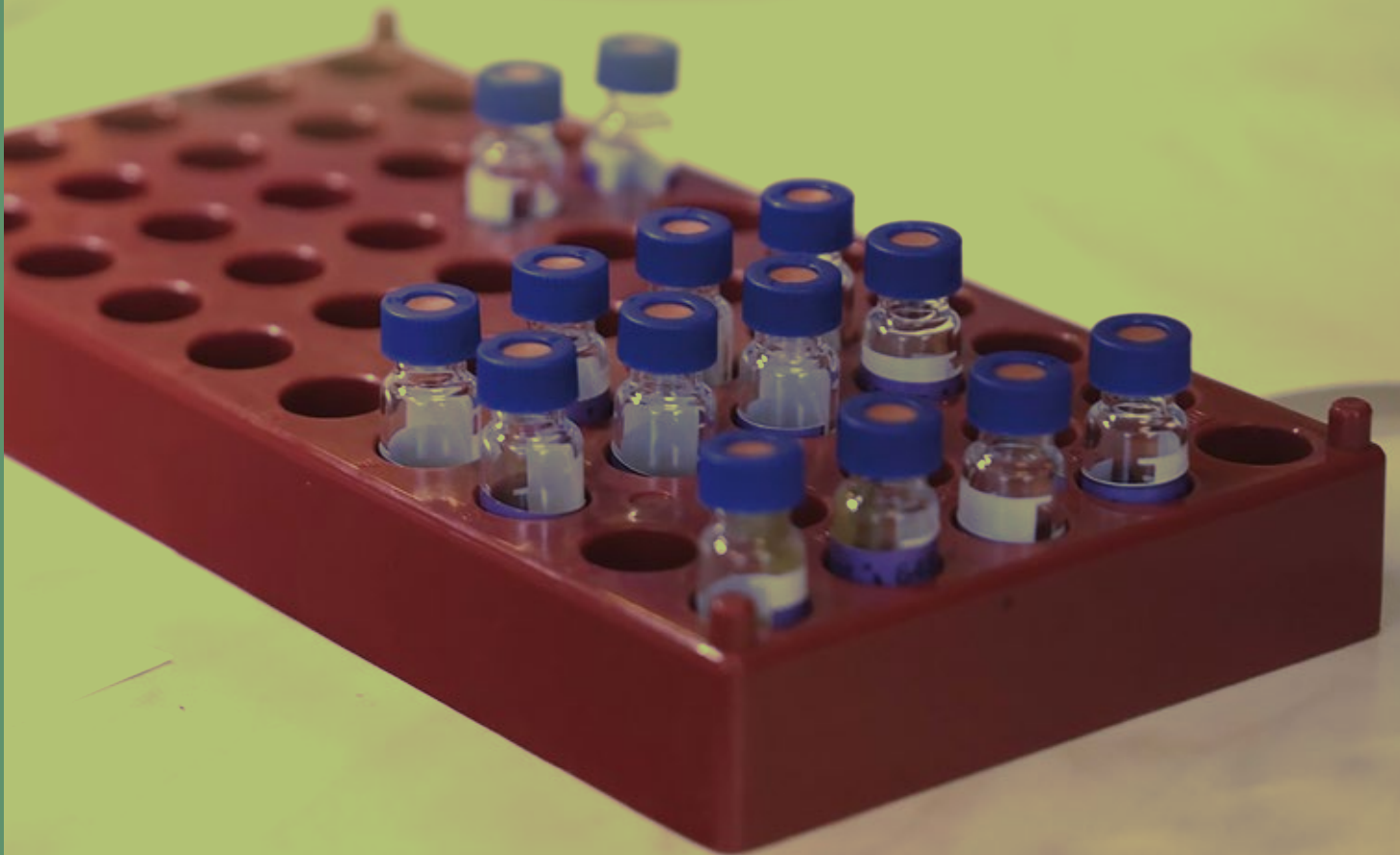
Domestication entails phenotypic changes driven by human selection, often accompanied or followed by diversification to meet diverse human uses and agronomic conditions. Similar domestication and diversification traits can arise in closely related species through parallel evolution, or in distantly related species via convergent evolution¹. These phenotypic traits result from alterations in conserved genetic networks or biosynthetic pathways. Accordingly, analogous traits may be governed by homologous genes (genetic parallelism) or by distinct, non-homologous genes (genetic convergence). Domestication is recognized as a selection-driven process - primarily human, but also natural - that increases the frequency of traits advantageous under cultivation. These traits comprise the domestication syndrome, including increased organ size, loss of natural dispersal mechanisms, altered plant architecture, and reduced seed dormancy. The study presents a comparative analysis of independently domesticated legume species from two regions of crop origin: chickpea, lentil², and pea³ from the Middle East, and common bean⁴ from Central America. It focuses on two critical traits: loss of seed dormancy and changes in pod dehiscence. By examining these traits across phylogenetically distinct legumes, the study explores whether similar phenotypic outcomes result from parallel or convergent genetic mechanisms⁵. Understanding the molecular basis of these traits not only elucidates the evolutionary processes underlying domestication but also informs strategies for modern crop improvement. As global agriculture faces increasing challenges, insights from domestication research offer valuable tools for breeding more resilient and sustainable crop varieties.

Acknowledgement

Work is supported by LUAUS25035 project from MEYS CZ. References to our work: ¹Smýkal et al. (2018) doi.org/10.3390/agronomy8030026, ²Guerra-Garcia et al. (2024) [doi:10.1002/tpg2.70021](https://doi.org/10.1002/tpg2.70021), ³Klčová et al. (2024) [doi:10.1111/tpj.16734](https://doi.org/10.1111/tpj.16734), ⁴Parker et al. (2021) doi.org/10.1093/plcell/koaa025, ⁵Yong, Balarynová et al. (2024) doi.org/10.1093/gbe/evae267.

Session 5:

**Plant proteomics & metabolomics
and plant biotechnology**



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Key words: chronic ionizing radiation, *Phragmites australis*, posttranslational regulation, protein carbonylation, proteome profiling

Chronic ionizing radiation causes elevated levels of DNA damage and reactive oxygen species in plants, considerably affecting their growth and development. Aquatic ecosystems in Chornobyl zone, a major radiological disaster site, are contaminated by harmful radionuclides. We focused on explaining the biochemical mechanisms responsible for the susceptibility of a wild aquatic plant (common reed, *Phragmites australis*) grown in Chornobyl zone to biotic stress. The fungal infection assay indicated that life in a radionuclide-contaminated environment might compromise plant immunity. Upon proteome profiling we selected several dozen proteins with consistently higher and lower abundance in the samples from the littoral of contaminated lakes by hierarchical clustering. Of note, we showed a major influence of confounding environmental variables on the reed proteome compared to radionuclide contamination. Proteins that accumulated in reed upon chronic irradiation suggested a biochemically stable phenotype with effective protection against reactive carbonyls. Simultaneously, proteins that depleted in plants collected from the littoral of radiologically contaminated lakes indicated worse stress resilience and enhanced susceptibility to biotic agents. Discordant expression of coding genes pointed to posttranscriptional regulation. Furthermore, the quantification of antioxidant enzyme activities and carbonylated proteins rebutted the idea of substantial oxidative stress in chronically irradiated plants. We propose that a formal assessment of safety in radionuclide-contaminated areas should weigh the risks of lower plant resistance to biotic agents.

Acknowledgement

This work was supported by the projects APVV-20-0545 and VEGA 2/0106/22.

COMPARATIVE TRANSCRIPTOME/PROTEOME ANALYSES REVEAL KEY PATHWAYS RESPONSIVE TO MANGANESE STRESS IN AQUATIC PLANT *SPIRODELA POLYRHIZA*

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Key words: duckweed, phytoremediation, proteome, stress response, transcriptome

Aquatic duckweed plants have great potential for phytoremediation due to their ability to remove ammonium, nitrates, phosphates, heavy metals, and organic xenobiotics from different types of wastewater. Manganese Mn, one of the essential micronutrients for eukaryotes, is toxic in high concentrations for plants, animals, and humans. In this work, we studied the response of great duckweed *Spirodela polyrhiza*, the prevalent duckweed species in Europe, to the elevated concentration of Mn by comparative characterization of transcriptome and proteome patterns. The duckweed molecular responses were analyzed in relation to the source of nitrogen, considering that compared to nitrate, ammonium alleviated Mn stress symptoms in duckweeds. A total of 5381 Mn-stimulated differentially expressed genes (DEGs) were revealed by transcriptome analysis in duckweed grown on nitrate-containing medium. In contrast, when duckweed was grown on the mixture of nitrate and ammonium supplemented with high Mn,

the number of DEGs was about 10-fold less, confirming the alleviating effect of ammonium. Among the most abundant DEGs were genes involved in oxidative stress, biotic and abiotic defense response, and hormone signal transduction. The proteomics data generated using a similar set of duckweed samples generally agreed and supported the transcriptomics results. The most representative among the revealed differentially accumulated proteins were proteins related to RedOx processes and oxidative stress, pathogenesis and defense response, and amino acid biosynthesis. The presented data shed new light on plant responses to heavy metal stress, revealing the major pathways involved in the response processes and paving ways for further investigations and application of these gene networks for engineering crops with improved stress tolerance.

UNVEILING NOVEL PROTEINS GOVERNING OXIDATIVE STRESS RESPONSE IN ARABIDOPSIS

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Key words: abiotic stress, proteomics, reactive oxygen radicals, protein networks, RACK1A

Reactive oxygen species accumulation induces a complex network of signaling events leading to the expression of a wide array of genes responsible for defense or repair mechanisms. Nevertheless, the protein networks responsive to oxidative stress are still far from being completely understood. We aimed to detect and characterize novel proteins contributing to oxidative stress resistance using integrative proteomic and cell biology approaches. A co-immunoprecipitation analysis combined with mass spectrometry suggested the RECEPTOR FOR ACTIVATED C KINASE 1a (RACK1a) to interact with IRON SUPEROXIDE DISMUTASE 1 (FSD1). RACK1a regulates FSD1 activity while both proteins accumulate in stress granules during salt stress response. They jointly contribute to plant salt stress resistance by controlling ROS accumulation. A shotgun proteomic analysis of hypersensitive Arabidopsis *fsd1* mutants uncovered remarkable protein networks responsive to early response to methyl viologen (MV) induced oxidative stress. The oxidative stress hypersensitivity was connected with perturbation in the abundance of chloroplast metabolic proteins, components of both photosystems and peroxisomal and ribosomal proteins in response to short-term MV treatment. FSD1 deficiency hindered the change in abundance of PATELLIN 4, a plasma membrane-localized lipid transfer protein. Further genetic, biochemical and microscopic analyses suggested that PATELLIN 4 contributes to early plant oxidative stress response. In conclusion, integrative proteomics approaches are powerful for the identification of novel protein players in controlling plant oxidative stress response.

Acknowledgement

This research was funded by a project LUAUS25235 from the Ministry of Education and Youth, Czechia and student project IGA_PrF_2025_020 from Palacký University Olomouc.

DROUGHT SENSITIVITY AND METABOLIC ADAPTATIONS TO DROUGHT STRESS: A CASE STUDY IN SEEDLINGS OF A NON-MODEL TREE SPECIES

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Key words: black pine, forest tree species, metabolomics, phenotyping, transcriptomics

Drought stress is a major factor contributing to the increasing mortality of tree species across Europe. The rapid pace of climate change is likely to outstrip the ability of forest tree species to adapt naturally. Understanding intraspecific diversity in drought responses is therefore crucial for both ecologically and economically important tree species. This knowledge can help ensure their continued contribution to ecosystem services and support the selection of more drought-resilient individuals for future breeding programs. We used high-throughput phenotyping with targeted and untargeted metabolomics and transcriptomics to investigate drought responses across climatically distinct populations of widely distributed Black pine (*Pinus nigra* Arnold s.l.) to assess the natural variation in drought sensitivity across nine black pine populations, and to investigate associated metabolic signatures in a subset of contrasting populations. Our results revealed significant variation in drought sensitivity among black pine populations. Climate–trait associations showed a weak, marginally significant correlation between aridity index and drought sensitivity. To investigate the metabolic basis of this variation, we selected four provenances (two tolerant, two sensitive). Targeted analysis confirmed known drought-related metabolites, whereas untargeted metabolomics showed that sensitive and tolerant populations clustered separately based on their metabolic profiles, indicating distinct drought-response strategies. Population-specific differences in gene expression related to these metabolites were further highlighted by transcriptomic profiling. Overall, our study reveals substantial natural variation in drought sensitivity among Black pine populations, which is associated with distinct metabolic profiles. The putative metabolites underlying distinct metabolic profiles offer promising candidates for future screening across broader populations or related conifer species.

Session 6:

**Phytohormones and root
& shoot development**

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Key words: biosynthesis, cytokinins, ethylene, multistep phosphorelay, signaling

The interconnected action of cytokinins and ethylene in the control of plant growth was demonstrated. However, the extent of the crosstalk and the underlying molecular mechanisms remained mostly elusive. We and others' have demonstrated that multistep phosphorelay (MSP) pathway, previously thought mainly to mediate cytokinin signaling, is under control of ethylene through the histidine kinase (HK) activity of ethylene sensor ETR1. Here we show that although ETR1 is an active HK, its receiver domain (ETR1_{RD}) is structurally and functionally unable to accept the phosphate from the phosphorylated His in the ETR1 HK domain (ETR1_{HK}) to initiate the phosphorelay to AHPs, the next downstream link in MSP signaling. Instead, ETR1 interacts with another HK AHK5 and transfers the phosphate from ETR1_{HK} through the receiver domain of AHK5 (AHK5_{RD}), and subsequently to AHP1, AHP2 and AHP3, independently of the HK activity of AHK5. We show that AHK5 is necessary for ethylene-initiated, but not cytokinin-initiated, MSP signaling *in planta* and is involved in the hormonal control of root growth and architecture in *Arabidopsis*. Furthermore, we have identified novel mechanism of transcriptional regulation based on the interaction of members of ethylene and MSP signaling pathways in the spatial-specific control of cytokinin-induced ethylene biosynthesis, mediating the cytokinin-induced, ethylene-regulated root growth. We propose that the aforementioned regulatory network represents a molecular basis for the existence of previously proposed morphogenic field combining the properties of both cytokinins and ethylene, controlling, together with auxin, root growth and patterning.

Acknowledgement

Supported by MEYS (CZ.02.01.01/00/22_008/0004581 and LUAUS24277).

ROLE OF THE AUXIN RESPONSE FACTOR5 AND MICRORNA390 IN EMBRYOGENIC TRANSITION IN *ARABIDOPSIS THALIANA*

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Key words: auxin, somatic embryogenesis, MONOPTEROS, YUCCA genes

Somatic embryogenesis (SE) is a process through which somatic plant cells acquire the capacity to initiate an embryonic developmental program. This remarkable form of cellular reprogramming is widely used in plant biotechnology for mass micropropagation, biodiversity preservation, and the production of transgenic plants. Despite its broad applications, the molecular mechanisms underlying SE initiation remain incompletely understood.

The plant hormone auxin plays a central role in SE, acting through key transcriptional regulators such as AUXIN RESPONSE FACTOR 5 (ARF5), also known as MONOPTEROS (MP). Auxin signaling, transport, and metabolism are essential for embryogenic reprogramming in *Arabidopsis thaliana* and other plant species. MP is strongly expressed during SE, and loss-of-function mutants show impaired embryogenic potential, highlighting its critical role.

Our study focuses on the function of MP11ir, a splice isoform of ARF5 characterized by intron retention. This alternative transcript encodes a truncated MP protein lacking the PB1 domain necessary for dimerization with Aux/IAA proteins, making it potentially insensitive to auxin repression. We found that MP11ir accumulates significantly during both auxin-dependent and auxin-independent SE induction. Functionally, MP11ir partially rescues the embryogenic potential in the *mps319* mutant background. However, overexpression of Δ ARF5, the truncated MP protein, disrupts somatic embryo formation and promotes callus development instead. This phenotype is associated with altered expression of auxin biosynthesis genes, resulting in disturbed auxin homeostasis and disrupted local and global auxin levels.

Taken together, this project aims to unravel the regulatory interplay between ARF5, auxin biosynthesis (via YUCCA genes), and auxin signaling (via MIR390) in controlling SE. Through a multidisciplinary approach—including CRISPR/Cas9 mutagenesis, MIR390 sensor development, dual-luciferase assays, gene expression profiling, hormonal analysis, and the green-CUT&RUN technique—we seek to clarify how ARF5 and its isoforms coordinate embryogenic transitions. These findings will contribute to a better understanding of auxin-driven developmental plasticity and improve protocols for plant regeneration and genetic transformation.

Acknowledgement

This work was supported by EXBIO (No. CZ.02.1.01/0.0/0.0/16_019/0000738) and the Polish National Agency for Academic Exchange (BPN/BEK/2021/1/00278/U/00001).

UNRAVELING THE RELATIONSHIP BETWEEN ROOT ELONGATION AND CELL WALL PH

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Key words: auxin, root, cell wall pH

The acid-growth hypothesis proposes that plants achieve cell elongation via apoplastic acidification, a process downstream of auxin signaling. However, how auxin precisely modulates cell-wall pH to drive turgor-driven expansion remains unclear. Intriguingly, auxin-transport-deficient mutants—despite altered surface pH—do not exhibit impaired elongation, suggesting a more nuanced relationship. To resolve this paradox, we engineered a genetically encoded, ratiometric pH sensor targeted to cellulose microfibrils, enabling direct, *in vivo* measurement of the cell-wall pH landscape and overcoming the limitations of surface assays. Deploying this probe in *Arabidopsis* roots, we mapped longitudinal pH gradients and uncovered unexpected alkaline domains within the elongation zone—directly contradicting classical acid-growth predictions. By integrating these data with high-resolution particle-image velocimetry (PIV), we compared local pH dynamics with precise elongation rates. Our results reveal a multifaceted interplay between apoplastic pH and cell elongation, challenging simplified models of acid-driven growth and highlighting the need for revised mechanistic frameworks.

Session 7:

**Advances in microscopy
and analytical techniques in plants**

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Key words: crops, light-sheet fluorescence microscopy, plants, super-resolution microscopy

Light-sheet fluorescence microscopy (LSFM) and super-resolution microscopy (SRM) methods are very important for modern plant research (Ovečka et al. 2022). Plants, especially crops, are quite difficult samples for microscopic methods due to the optical properties of their organs, tissues and cells. Moreover, they require specific illumination and perfusion systems to secure undisturbed plant growth and development as well as interaction with microbes in imaging chambers. Classical microscopy approaches have some critical limitations, like phototoxicity induced by high rate of irradiation, excessive out-of-focus fluorescence, low scanning speeds, limited imaging depth, mechanical and gravitational stress induced by plant mounting and artificial positioning in the microscope. LSFM and SRM methods can overcome some of these limiting factors. In the last decade we developed two robust and broadly applicable protocols for short-term and long-term bioimaging of living plants using SRM (Komis et al. 2015) and LSFM (Ovečka et al. 2015). We have used them for diverse purposes in plant biology research including studies showing contribution of secretory vesicles to the oscillatory growth of root hairs, signalling by mitogen-activated protein kinase during cell division, monitoring of microtubule dynamics during plant morphogenesis, dynamic behaviour and interactions of endosomes in root hairs and nuclear changes in diverse root developmental zones and tissues in *Arabidopsis*. We also developed custom-made illumination and perfusion systems allowing long-term multiscale imaging of crops such as alfalfa and barley as well as their interactions with microbes and studies focused on abiotic and biotic stresses (Ovečka et al. 2020, 2022; Hlaváčková et al. 2023).

Acknowledgement

Research was supported by program INTER-EXCELLENCE II (grant LUAUS25235) from the Czech Ministry of Education, Youth and Sports, Czechia and IGA_PrF_2025_020 Role of annexins ANN1, ANN3 and ANN4 in *Arabidopsis* root hair development.

ADVANCING PLANT METABOLIC RESEARCH - VALIDATED APPROACH FOR SIMULTANEOUS ETHYLENE, PHYTOHORMONE AND POLYAMINE DETECTION

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Key words: ethylene, mass spectrometry, photo-acoustic detection, phytohormone; polyamine

The phytohormone ethylene and polyamines represent critical signaling molecules governing plant developmental processes and stress adaptation mechanisms, with their biosynthetic pathways exhibiting significant interconnectivity through the shared Yang cycle. Despite their fundamental physiological importance, accurate quantification of these metabolites presents a substantial analytical challenge. We have created novel and validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) based approach, that enables (from the same biological sample) ethylene detection followed by simultaneous determination of 15 phytohormonal compounds, including auxins, cytokinins, jasmonic acid, abscisic acid, salicylic acid, and 1-aminocyclopropane-1-carboxylic acid, alongside 14 key metabolites from polyamine and amino acid pathways, such as methionine, putrescine, spermidine, spermine, thermospermine, L-arginine, L-citrulline, and L-ornithine. The methodology has been optimized for small sample quantities, requiring only 10 mg of fresh-weight plant tissue. Implementing this analytical approach on *Arabidopsis thaliana*, its ethylene biosynthesis mutants and other plant species (*Solanum lycopersicum*) at the seedling stage has uncovered differential metabolic responses. Drought and salt stress induce distinct alterations in the accumulation patterns of polyamines and ethylene



precursors, with spermine demonstrating particularly pronounced stress-specific changes in concentration. Additionally, impaired ethylene biosynthesis in *Arabidopsis* affects specific phytohormones, such as auxin, while having minimal effect on others.

COMPARATIVE METABOLITE PROFILING APPROACH REVEALS THE COMPLEXITY OF AUXIN METABOLISM ACROSS PLANT SPECIES

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Key words: auxin metabolism, mass spectrometry, metabolite profiling, plant hormones

Auxin plays a pivotal role in plant growth and development, with its metabolism tightly regulated to ensure precise spatial and temporal regulation of signaling. While the canonical pathways of auxin biosynthesis, transport, and degradation - particularly those involving indole-3-acetic acid (IAA) - are well established in *Arabidopsis*, emerging evidence points to a much greater complexity and evolutionary diversity in auxin metabolism.

To investigate this, we developed novel approach combining micro-scale extraction with ultra-sensitive liquid chromatography - mass spectrometry (LC-MS), enabling detailed profiling of auxin metabolites across diverse plant species. Applying this method to *Arabidopsis thaliana* and selected crop plants, we identified novel endogenous auxin metabolites and observed species-specific and tissue-specific variations in metabolite profiles. Notably, auxin metabolite levels were found to be strongly dependent on developmental stage, underscoring the dynamic regulation of IAA metabolism during seedling growth.

Furthermore, an in-depth analysis of phenylacetic acid (PAA) metabolism revealed that IAA and PAA share a conserved core metabolic framework, indicating a broader and more integrated network of auxin regulation than previously appreciated. These findings enhance our understanding of auxin metabolic networks and underscore the intricate regulatory mechanisms that underpin plant hormonal balance.

Acknowledgement

The work was supported by the project TowArds Next GENERation Crops (TANGENC), reg. no. CZ.02.01.01/00/22_008/0004581, of the ERDF Programme Johannes Amos Comenius and by the ERC Synergy project STARMORPH (No. 101166880) under the European Union's Horizon Europe research and innovation program.

IN SITU COMPARISON OF ABIOTIC AND BIOTIC INDUCED PHYTOHORMONE CHANGES USING MASS SPECTROMETRY IMAGING

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Key words: abiotic, biotic, mass spectrometry imaging, phytohormone

As one of the most important signalling biomolecules, phytohormones, together with their metabolites, play critical roles in regulating plant responses to different stresses, such as physical damage or microbial invasion. However, direct visualisation and *in situ* identification of multiple phytohormones from their original localisations are still challenging tasks due to their very low abundance and complex molecular backgrounds inside plant tissues.

Currently, liquid chromatography-mass spectrometry (LC-MS) and reporter gene assays are predominant methods for the targeted phytohormone analysis in plants, but spatial distributions of untargeted phytohormones and compounds involved in the metabolomics network cannot be measured within the same experiment. On the other hand, mass spectrometry imaging (MSI) as an untargeted and label free analytical method has been widely applied for the *in situ* visualizations of plant metabolites, lipids, proteins as well as other biomolecules, and has demonstrated unique advantages in comparison with classical analytical methods (Lee et al., 2012). Recently, we have applied desorption electrospray ionisation mass spectrometry imaging (DESI-MSI) to visualise multiple phytohormone species and related derivatives from leaves and roots (Zhang et al., 2021; Zhang et al., 2024).

Aiming to further compare plant hormonal alternations between abiotic and biotic stress responses, DESI-MSI coupled with ion mobility (IM) was performed on *Arabidopsis* leaves damaged *via* physical wounding and pathogenic bacteria wounding to characterise major phytohormones as well as other related metabolites from their intact positions. Our results highlighted unique distributions of salicylic acid, auxins, jasmonic acid, and cytokinins from stressed leaves. Further correlation analysis reveals potential spatial connections among phytohormone species and other detected metabolites. This suggests that DESI-IM-MSI not only enables high throughput analysis of phytohormones in plants under biotic or abiotic stress, but also offers insights into hormone-hormone and hormone-metabolite interactions within heterogeneous plant tissues.

Acknowledgement

The work was supported by Czech Science Foundation (no. 24-11511S).

Session 8:

Plant adaptation to abiotic stress, phytoremediation and phytotechnologies

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Key words: *Callitriche* sp., chromium, homeostasis, phytoremediation, water

Water-starworts (*Callitriche* sp.) are common, higher aquatic plants found in temperate climates worldwide, able to remove many dangerous metallic elements from aquatic systems. In research conducted over several years at the Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Poland, we have demonstrated that *Callitriche cophocarpa* L. exhibits an exceptional potential for phytoremediation of waters contaminated with highly toxic chromium ions.

The plant's shoots are capable of hyperaccumulating chromium to a level of c.a. 30 mg/g d.m. ("normal plants" accumulate only a few µg/g d.m.). The maximum sorption capacity of the biomass reaches 77 mg Cr(III)/g d.m., which is even higher than that of some commercially used sorbents. Plants effectively detoxify the chemically aggressive form Cr(VI) by reducing it to the less toxic Cr(III). The reduction takes place under the control of the FQR1 dehydrogenase. The distribution of Cr in tissues depends on the speciation of Cr, which affects the plant. Consider the genus's above-average physiological tolerance to abiotic stress, we are currently creating the first world's *in vitro* collection of different genotypes – species and ecotypes of water-starworts, for the model studies on metal homeostasis mechanisms in aquatic plants.

C. cophocarpa also acclimatizes very easily after introduction into harsh environmental conditions. Additional auto-inoculation of the plants with their bacterial symbionts seems beneficial before introducing the shoots into polluted water. *Callitriche* sp. can be used in constructed wetlands fed with chromium. Due to the limited capacity of the shoots to remove Cr(III) from bottom sediments, in a constructed wetland for simultaneous remediation of water and sediments, it is advisable, together with *Callitriche* sp., to use other species rooted in the substrate: *Epilobium obscurum*, *Alisma plantago-aquatica*, and *Veronica beccabunga*. The pilot-scale constructed wetland for Cr removal from industrial effluents at the premises of a chemical company will be presented.

Acknowledgement

This research was funded in part by the National Science Centre, Poland, grant Opus 28 number 2024/55/B/NZ8/01640.

NITRIC OXIDE SUSTAINS ROOT SURFACE REDOX ACTIVITY AND GROWTH DURING FLOODING STRESS IN BARLEY ROOT TIP

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Keywords: barley, hypoxia, nitric oxide, root surface redox activity

Climate projection models suggest an increase in frequency and severity of sudden flooding events in farming regions, which will limit plant growth and development by altering numerous morphological, physiological and biochemical processes; in very sensitive plants they may even evoke cell death. In order to gain more insight into the plant response to sudden flooding-induced hypoxic stress, we studied the involvement of an important signaling molecule - nitric oxide (NO), in various barley cultivars (cv.) at their early seedling stage during short-term partial submergence-induced stress. Sudden flooding stress induced a root growth arrest in cvs. Karmel and Levitus, accompanied by increased lipid peroxidation and cell death. By contrast, in more flooding-tolerant cvs. (Slaven and Valis) sudden flooding caused only reduced root growth rate, associated with elevated NO levels in the root tips. Meanwhile, the root tip surface redox activity decreased with the increasing timespan of flooding in all cvs.; however, this decrease in redox activity started earlier and was greater in the cvs. Levitus and Karmel in comparison with cvs. Valis and Slaven. Application



of NO donors, sodium nitroprusside and S-Nitroso-L-glutathione, during flooding stress restored the root redox activity and eliminated the flooding-induced lipid peroxidation, accompanied by a partial restoration of post-flooding root growth even in the more sensitive cultivars. This indicates that NO plays a key role in maintaining the root tip surface redox activity in barley seedlings under sudden flooding stress, which is necessary to restrict lipid peroxidation and cell death in the root tips.

Acknowledgement

This work was supported by the Grant Agency VEGA, project No. 2/0059/24.

STRUCTURAL ASPECTS OF PLANT REACTIONS TO STRESS

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Key words: abiotic stress, adaptation

Land plants are continuously exposed to stress and due to their sessile type of life they developed a scale of adaptation. Extremophiles even use various harsh conditions to exist in areas with limited competition of other organisms. Physiological and molecular mechanisms of these adaptations are intensively studied in various species. In the present contribution I will try to summarize some interesting aspects of structural adaptations and reactions to stress. The results represent outcomes of long lasting microscopical analysis of our team. Plant reactions to abiotic stress as drought, salinity, nutrient imbalance, metal and metalloids toxicity and some biotic stresses will be introduced and compared with recent published data. The reactions at the level of whole plant body, plant organs, mostly roots, specific tissues and cells will be shown. The analysed species include some model plant species but mainly crops and some woody species. The main outcome of these observations is that plants are smart and that still many of their reactions to various stress conditions are not well understood.

Acknowledgement

My acknowledgement belongs to many current and former colleagues and friends from our department and from numerous laboratories in several countries which I have visited. The recent work was financially supported by the Slovak Research and Development Agency under contract Nr. APVV-17-0164, and VEGA1/0472/22.

DIFFERENCES IN THE PRODUCTION OF ROOT EXUDATES AND PHYSIOLOGICAL RESPONSES OF C3 AND C4 CROPS TO DROUGHT STRESS AND NITROGEN FERTILIZATION

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Key words: C3-crops, C4-crops, drought-stress, root-exudation

Root exudates are central in shaping plant–soil–microbe interactions by mediating nutrient availability, microbial activity, and chemical signaling within the rhizosphere. Although they typically represent less than 0.4% of photosynthetically fixed carbon, root exudates include a chemically diverse mixture of compounds, including amino acids, organic acids, phenolics, and other secondary metabolites, that can significantly influence belowground processes. This study investigates root exudation profiles and physiological responses in two C3 crops (barley and quinoa) and two C4 crops (maize and amaranth)

subjected to contrasting nitrogen fertilization and water availability conditions. Plants were grown under controlled environmental conditions using a drip irrigation system to ensure a consistent water supply during the early growth phase. Nitrogen fertilization began after the third leaf stage and was maintained throughout the experiment in well-watered treatments but discontinued in drought-stressed treatments upon initiation of water restriction. Drought stress was applied four days before measurement by withholding water to simulate acute moisture limitation. Root exudates were collected over 24 hours by immersing plant roots in distilled water and were subsequently analyzed using high-performance liquid chromatography coupled with mass spectrometry. In parallel, physiological parameters including photosynthetic rate, stomatal conductance, and chlorophyll fluorescence were measured to assess drought and nitrogen responses. Although data analysis is still in progress, the study is designed to reveal how nitrogen and water availability influence root exudation and physiological traits in crops with distinct photosynthetic pathways. For example, vanillic acid was identified as a dominant exudate in C3 crops such as quinoa and barley, whereas chlorogenic acid was higher in the C4 crops (amaranth and maize). Among all detected exudates, proline showed one of the highest increases under drought conditions. These findings will support a broader understanding of how root exudates contribute to rhizosphere ecology and crop resilience under variable environmental conditions.

Acknowledgement

The Ministry of Education, Youth and Sports of the Czech Republic financially supported this research through the AdAgriF project (C Z.02.01.01/00/22_008/0004635).

NITROGEN AND OTHER ABIOTIC FACTORS AFFECTING ISOFLAVONOID AND FLAVONOID PRODUCTION IN *LOTUS SP.*

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Key words: flavonol, glycosylation, isoflavanes, malonylation, nitrate

Nitrogen is a crucial plant macronutrient involved in the balance between primary metabolism and the flux toward secondary metabolic pathways. Low nitrogen availability has been found to increase the accumulation of phenolic compounds in plants. A high C/N ratio enhances phenylalanine ammonia lyase (PAL) activity, which releases ammonia from the phenylalanine substrate and increases available nitrogen in plants. This study investigated the effect of different nitrogen conditions on the production and accumulation of phenolic compounds in leaves of *Lotus corniculatus*. Low nitrogen conditions strongly enhanced phenolic biosynthesis in leaves, inducing the expression of genes encoding key enzymes of phenolic metabolism: phenylalanine ammonia lyase, chalcone synthase, and isoflavone synthase. The involvement of different isogenes in these biosynthetic pathways will be discussed. Kaempferol and quercetin glycosides, as well as isoflavones, accumulated in leaves under low nitrogen conditions. Nitrogen limitation may be one factor affecting quercetin levels. Surprisingly, when nitrogen availability was limited but without causing nitrogen deprivation symptoms, an increase in phenylpropanoid levels and a decrease in kaempferol glycoside levels were observed. The decrease was particularly apparent for malonylated kaempferol derivatives and kaempferol glycosides substituted by rhamnose at the 3-O position. The involvement of UGT genes in these differences remains unclear. The *L. corniculatus* malonyltransferase 1 gene (*LjMaT1*) is likely involved in the malonylation of kaempferol glycosides

Acknowledgement

The authors acknowledge the funding provided by VEGA 1/0452/24 from the Ministry of education, science, research and sport of the Slovak Republic, Project PID2021-122353OB-I00 (MCIN/AEI/10.13039/501100011033/ and FEDER Una manera de hacer Europa).

IMPACT OF HEAT WAVES ON SEED DEVELOPMENT IN *ARABIDOPSIS THALIANA* AND *BRASSICA NAPUS*

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Key words: *Brassica napus*, heat stress, seed coat integrity, seed development

In recent decades, we have witnessed the global effects of climate change with increases in average ambient temperatures and the frequency of heat waves. As a result, crop yields in temperate regions have declined and are expected to decline further in the coming years. In flowering plants, the reproductive phase is a developmental stage sensitive to high temperatures. The response to high temperature involves transcription factors such as phytochrome interacting factors or heat shock factors, chaperone proteins, and phytohormone production, creating a complex response with multiple levels of regulation. Using the tools available at the CEITEC Plant Sciences and Cellular Imaging Core Facilities, we investigated seed development in *Arabidopsis thaliana* and *Brassica napus* plants under different high temperature scenarios. Our results provide new data on long-term adaptation to high temperatures during the seed production phase. Reduced fertility leads to reduced seed production and seed quality in both species. In *Brassica napus*, high temperatures above the optimum growth temperature resulted in the production of seeds with ruptured seed coat, a phenotype associated with accelerated embryo development. While the relationships between high temperature, embryo growth rate, and seed coat rupture remain unclear, our data provide novel observations to address the issues linking high temperature responses to seed biomechanical properties. Our work provides insight into the effects of high temperature on seed production and opens the door to a more detailed analysis of the molecular mechanisms responsible for these effects.

Acknowledgement

Funding sources: GA22-29717S by Czech Science Foundation, TANGENC (TowArds Next GENeration Crops) CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes Amos Comenius by MEYS.

EFFECT OF METALS AND METALLOIDS ON GROWTH OF VARIOUS SPECIES AND ECOTYPES FROM SALICACEAE FAMILY

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Key words: fast growing trees, metals and metalloids, phytoremediation, willows and poplars

Trees from Salicaceae family, especially willows (*Salix* sp.) and poplars (*Populus* sp.) have been shown as suitable plant species for remediation of contaminated sites and substrates due to their fast growth and relative large biomass production. While their capacity for uptake of various contaminants is considerably lower than in hyperaccumulators, they might be rather used in production of safe wood biomass. Phytostabilisation of contaminated sites using tolerant species and varieties of plants is nowadays considered as a suitable, and in many cases, more effective strategy for restoration of contaminated sites than phytoremediation. However, it is necessary to select a suitable plant species or ecotypes with optimal growth



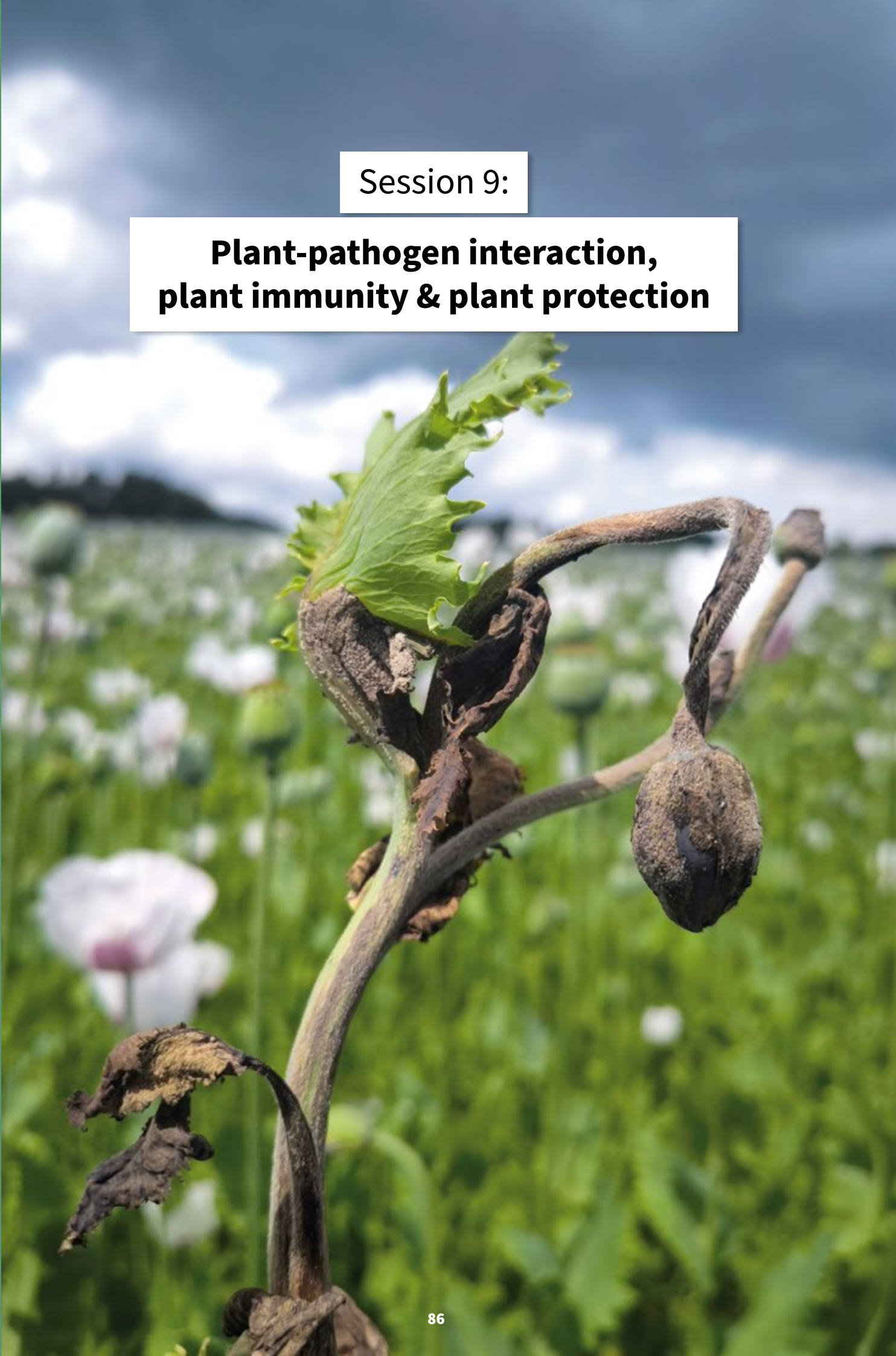
properties before they will be sown in real conditions at the sites. In the recent study we investigated several variable species and ecotypes of willows (*Salix* sp.) and poplars (*Populus* sp.) and compared their response to elevated concentration of various metals and metalloids in hydroponics. To address whether particular species and ecotypes are more suitable for extraction of metals or might be rather used for phytostabilisation of metal(loid) contaminated soils we evaluated the biomass production, quantum yield of photosystem II and chlorophyll content as well as uptake of contaminants in plants aboveground tissues.

Acknowledgement

This work was supported by the Slovak Research and Development Agency (grant numbers APVV-23-0318, and APVV SK-TW-24-0008), and by Slovak Grant Agency VEGA (grant number VEGA 2/0047/25).

Session 9:

**Plant-pathogen interaction,
plant immunity & plant protection**



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Key words: flavonoids, microbial symbiosis, pathogen resistance, pea (*Pisum sativum*), *Didymella pinodes*

Plants react to multiple abiotic and biotic influences. Therefore their response need to be differential. Friends need to be distinguished from enemies. Nevertheless, plants also need to keep their symbiotic partners in check. Hence, microbial interaction partners can prime the immune system of the plants such that they may also be better protected against pathogens.

Ascochyta blight, caused by the fungal pathogen *Didymella pinodes*, is a significant threat to pea cultivation. It can cause up to 80% yield loss. To date, no substantial plant resistance mechanism has been found to better tolerate or even prevent the disease.

With the example of pea plants (*Pisum sativum*), I will talk about how different cultivars of the same plant species developed different molecular strategies to protect themselves from *D. pinodes*. Rather than the synthesis of one protective substance, the concerted production of several flavonoids seems to be a more successful strategy. Further, I will discuss whether/how microbial symbiosis induces systemic tolerance against those pathogens and what influence this may have on seed quality (and quantity).

Concerning pathogen resilience, I will finally also touch the challenging subject of increasing temperatures and possible changes in humidity.

PATTERN-TRIGGERED IMMUNITY IN *PAPAVER SOMNIFERUM*

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Key words: PTI, poppy, chitin, flg22, crop protection

Papaver somniferum (poppy) is a traditional component of Central and Eastern European cuisine and an important oilseed crop of this region. The important threat for poppy stable yield is pathogen infection. Thus, we need to understand poppy defence mechanisms in detail. The first robust layer of plant immunity, which plays a crucial role in combating pathogens, is pattern-triggered immunity (PTI). Here, we provide the first insights into PTI in poppy. We selected four poppy varieties widely used in the food industry. We investigated poppy response to various elicitors acting as microbe-associated molecular patterns (MAMPs) and damage-associated molecular patterns (DAMPs). Flg22 and chitin induced the most robust reactive oxygen species (ROS) burst among all tested elicitors. Flg22 and chitin also triggered putative mitogen-activated protein kinase (MAPK) phosphorylation and seedling growth inhibition in all tested cultivars. We identified genes which can serve as markers for monitoring poppy PTI responses. The tested poppy cultivars have low levels of salicylic acid. Callose accumulation was triggered by wounding but further elevated by tested elicitors. Our findings highlight conserved aspects of poppy immunity and challenges in studying poppy PTI. The established pipeline facilitates improving our understanding of poppy immunity and has the potential for widespread application in poppy breeding and improving selection for broad-spectrum disease resistance provided by enhanced PTI.

Acknowledgement

We would like to thank for financial support from MEYS Inter-Excellence II, Inter-COST project nr. LUC23146.

BATTLEFIELD - PLANT: EXPLORING THE DYNAMICS OF TRIPARTITE INTERACTIONS BETWEEN PLANTS, BACTERIA AND BACTERIOPHAGES

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Key words: bacteriophages, biocontrol, plant-microbe interactions, plant immunity, *Pseudomonas*

Bacterial diseases are responsible for major losses in vegetable crop production, and are particularly difficult to manage in close cultivation systems, aka greenhouses. While EU regulations still limit genome editing of plants, and in the context of increasing antibiotic resistance, bacteriophages (phages) appear as promising biocontrol agents. However, there are several limitations of their broad use, such as an unknown impact on plant-associated microbiome, fast evolution of the microbial community, and the lack of knowledge about impact on plants.

On the way of developing a functional biocontrol solution against bacterioses for greenhouse-cultivated tomatoes, we have optimized a pipeline of phage isolation, and now investigate the **tripartite interactions between phages, bacteria and plants** using the model system based on *Arabidopsis thaliana*, *P. syringae* pv *tomato* and *Pseudomonas* phage Eir4. We found that phages with simple morphology and lytic life cycle are capable to persist in plant tissues, and restrict bacterial propagation while applied before or after the infection. Pre-treatment with minimally purified phage preparations also had a priming effect on plant immune system, triggering a mild activation of defense responses (transcriptome remodeling, callose deposition, ROS production), and enhanced resistance to subsequent infection with virulent pathogen. At the bacterial side, phage presence stimulated resistance development in the microbial population, but often for a cost of virulence loss, thus still beneficial for practical application. Combining the advanced imaging, genomics, and plant phenotyping we aim to shed light on the mechanisms of phages action *in planta*, thus opening their use as efficient customizable biocontrol for sustainable agriculture.

Acknowledgement

The work is supported by Technological Agency of Czech Republic (TAČR, TQ03000088). IEB Imaging Facility is supported by a project of the MEYS grant LM2023050 Czech-Biolmaging". NK and OB received individual fellowships from the Visegrad Fund and FEMS. Cooperation between Czech and Latvian academies of Sciences is covered by a grant LZA-25-02.

HORMONAL CROSSTALK IN FUNGAL PATHOGENICITY: AUXIN, CYTOKININ AND SALICYLIC ACID OF *LEPTOSPHAERIA MACULANS*

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Key words: auxin, fungal pathogen, *Leptosphaeria maculans*, phytohormones, salicylic acid

Phytohormones are critical regulators of plant growth, development and defense. Pathogens like *Leptosphaeria maculans*, the causal agent of blackleg disease in *Brassica napus* (oilseed rape), exploit these signaling pathways to enhance virulence. This fungus synthesizes auxins, cytokinins (CKs), and salicylic acid (SA). Auxin production in *L. maculans* is induced by

precursors such as tryptophan and tryptamine, with transcriptional activation of biosynthetic genes *LmTAM1* and *LmIPDC2*. Exogenous auxin application modulates necrotic lesion size on host plants, while fungal auxin excretion suggests a direct role in pathogenesis. CK biosynthesis, mediated by isopentenyltransferase (*LmIPT*) and adenosine kinase (*LmAK*), is essential for fungal fitness; suppression of these pathways reduces virulence. Fungal CK profiles differ from the host, predominantly featuring free CK bases. *L. maculans* also produces SA, utilizing orthologues of plant biosynthetic genes like *AtICS1* or *AtPAL1-4* and employs an SA-sensing mechanism involving the responsive gene *LmSrg1*. The *LmSrg1* gene can also be induced by several bacterial strains and exogenously supplemented auxin precursors which suggests more complex role of the fungal SA signalling than sole interaction with the plant host. Using LC-MS we have identified potential SA biosynthetic and metabolic pathways involving chorismate, tryptophan and phenylalanine. Observed interplay between fungal SA and auxin signaling suggests complex hormonal manipulation during infection. This integrated view of fungal hormonal hijacking advances broader insights into plant-microbe interactions and pathogen evolution.

Session 10:

**Crop reproduction and nutrition,
biology of trees**

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Key words: functional foods, grain composition, nutrition, plants, primary food raw materials

Plants have long served as the basis of human nutrition, providing important macronutrients, micronutrients, and bioactive compounds as a source of energy and necessary substances as well as necessary for human health. To face global challenges such as malnutrition, population growth, climate change, sustainability, and circular economy there is a pressing need to enhance the nutritional value of primary food raw sources through a deeper understanding of the biological, environmental, and technological factors influencing their composition. This work explores the central role of crops as nutritional pillars, focusing on strategies to optimize grain composition for improved human health outcomes and agriculture as a tool for their production friendly to the environment. The key physiological, genetic, and environmental determinants that influence the grain nutritional profiles, including protein content, lipid quality, polysaccharides composition, are discussed, as well as the presence of phytochemicals with functional and biological values. Emphasis is placed on breeding techniques that allow for targeted improvements in crop quality traits. Selected biotechnological approaches, such as sprouting and fermentation, offer promising tools to enhance the acceptability of the raw material for the consumer by the accumulation of health-promoting compounds and the reduction of anti-nutritional factors. Furthermore, the concept of functional foods and the development of value-added crop products are examined in the context of consumer health and market demands. By integrating plant science, human nutrition, and food technology, this research advocates for a system-level approach to designing crops and foods prepared from them that not only meet caloric needs but also actively contribute to disease prevention and human well-being. Ultimately, reprogramming crop composition for human benefit requires a multidisciplinary effort involving plant breeders, plant biologists, nutritionists, and policy makers. This comprehensive approach supports the transition from staple crops as sources of sustenance to powerful tools for nutritional intervention and global health promotion.

Acknowledgement

The Slovak Research and Development Agency supported this research under contract no. APVV-23-0375.

BIOCHEMICAL RESPONSES OF SWEET CHERRY TO REPEATED WATER DEFICIT

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Key words: organic acids, prolin, sugars, sweet cherry, water stress

Drought is considered as important abiotic factors affecting plant growth and yield. Even in irrigated orchards, trees may be subjected to cyclically recurring periods of water stress and recovery. Adaptive mechanism to drought is a complex phenomenon and its understanding is essential for (i) the successful setting of water irrigation to ameliorate damage on fruit yield and quality, and (ii) optimizing the varietal selection in plant breeding. Six sweet cherry cultivars were grown in greenhouse in container with two irrigation regimes: control trees (100% Etc) and stressed trees subjected to two 14-day water withholding (50% Etc) separated with 14-day recovery periods. Drought induction (both periods) increased the levels of hydrogen peroxide and superoxide radical. Subsequent rehydration reduced these values, but they still remained higher compared to the control. Proline is considered an effective antioxidant and important osmoprotectant. Its level increased especially during the second drought period. The first drought



period increased the levels of sucrose, raffinose, stachyose and sorbitol mainly in sensitive genotypes (Jacinta, Justyna and Kordia). Rehydration reduced these values, with exception for sucrose, where values remained still high. Surprisingly, after second rehydration, higher values of raffinose and stachyose were recorded in both control and stressed plants. This may be due to overheating in the greenhouse, when plants prevent excessive water loss by increasing the production of osmotically active carbohydrates. This hypothesis is also supported by the reduction in sucrose at the expense of increased accumulation of fructose and glucose as the need for effective osmolytics. Dehydration led to an increase in the content of malic, citric and fumaric acids. Rehydration had no significant effect and the values remained high. More resistant genotypes are generally considered to be those with lower ROS production and higher proline content. This was also confirmed in our case with the tolerant genotypes Amid, Early Korvik and Regina.

Acknowledgement

The study was financially supported by the Specific Research Project of the Faculty of Science, University of Hradec Kralove, No. 2107/2023 and by Ministry of Agriculture of the Czech Republic, No. QK21010200.

EFFECT OF NATURAL-BASED BIOSTIMULANTS ON POPPY YIELD PARAMETERS

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Key words: alkaloid, biostimulants, poppy, stress, yield

Abiotic stressors, particularly drought, are causing increasing damage to agriculture. To mitigate the impacts of stress and enhance yields, biostimulants are increasingly being utilized. Poppy (*Papaver somniferum* L.) is a unique crop in the Central European region, particularly in Czechia, Slovakia and Hungary. The primary goal of poppy cultivation is the production of seeds, but in the case of high alkaloid content (industrial) varieties, the pharmaceutical industry also utilizes poppy straw. In our field experiments, we studied the effect of various plant or algae-based biostimulants on industrial poppy over a period of three years. Our results show that the effect of the tested biostimulants is dependent on weather conditions. Under moderately unfavorable weather conditions, biostimulants significantly contributed to yield increase. In contrast, in extremely dry years, the effect of biostimulants was lower, suggesting that precipitation conditions are important factors in the effectiveness of biostimulants in poppy cultivation. The treatments generally affected the concentration of alkaloids accumulated in poppy capsules only to a minor extent. However, the alkaloid production per unit area increased in many cases due to the increase of capsule dry mass.

Based on this, biostimulants can contribute to increasing poppy yields, provided that environmental conditions, especially water supply, are not extreme unfavorable. Our results indicate that adaptation to changing environmental factors – primarily drought – is essential when developing modern cultivation technologies. Further research is needed to refine the application of biostimulants in the face of a changing climate.

Acknowledgement

Project no. KDP-5-3/PALY-2022 has been implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, financed under the KDP-2021 funding scheme.

26th–28th August 2025

Plant Biology CS 2025



Posters

GENETIC ENGINEERING OF CZECH HOP CULTIVARS

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Key words: CRISPR, early flowering, genetic transformation, hop, regeneration

Common hop (*Humulus lupulus* L.), a perennial crop within the *Cannabaceae* family, is essential to the brewing industry. Hop has deep economic and socio-cultural roots in Central Europe, especially the Czech Republic, particularly because of the traditional 'Saaz' cultivar. However, rising global temperatures, prolonged summer heatwaves, and increased climatic instability are threatening both the yield and chemical composition of hop cones, underscoring the urgent need for climate-resilient cultivars.

To address these challenges, there is a pressing need for innovative approaches that can accelerate hop breeding and enhance its adaptability to climate change. Genetic improvement in hop remains mostly constrained by its long vegetative phase, limiting functional genomic analysis. We present an integrated strategy to induce early flowering to enable the effective use of New Genomic Techniques on hop. We optimize *Agrobacterium*-mediated transformation protocol and *in vitro* regeneration conditions to improve the efficiency of transgene delivery. Using this platform, we overexpress key floral activators - *FLOWERING LOCUS T (FT)* and *LEAFY (LFY)* - to stimulate a faster onset of flowering. We are also testing an alternative strategy - CRISPR/Cas9-mediated genome editing to knockout flowering repressors, such as *SHORT VEGETATIVE PHASE (SVP)* and *TERMINAL FLOWER (TFL)*, generating targeted mutant lines with shortened vegetative phases. Further, we investigate the effect of plant growth regulators and cultivation conditions *in vitro* on transformation outcomes.

Collectively, this work establishes a molecular toolkit for functional genomics in hop and provides a pathway toward the rapid generation of elite cultivars resilient to climate-related challenges in the future.

Acknowledgement

The work was supported from the project TowArds Next GENeration Crops, reg. no. CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes Amos Comenius.

HORMONAL PROFILES ASSOCIATED WITH THE DEVELOPMENT OF TURIONS (WINTER BUDS) OF AQUATIC PLANTS

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Key words: ABAs, auxins, CKs, dormancy, overwintering

Turions (overwintering buds) are dormant storage organs, modified shoot apices, found in many vascular aquatic plants. They separate from senescent mother shoots and overwinter at the bottom of water bodies. To understand the hormonal regulation of turion formation and maintaining turion dormancy over winter, we investigated the hormonal profiles of CKs, auxins, and ABAs in miniature samples of developing turions in two species and mature autumnal turions in 22 species, using UHPLC-MS/MS. These were compared with those in overwintered turions under imposed dormancy and with non-dormant winter shoot apices. The content of active CKs was either stable or decreased during turion development. In both

species, IAA content culminated in the middle of turion development and then declined again. As the main inhibitory hormone, the ABA content was nearly undetectable in growing plants but increased significantly during turion development, remaining very high in mature turions. In innately dormant turions of 22 species, the proportion of biologically active CK forms was 0.18–67% of the total content. Similarly, the IAA proportion as the active form was very variable (0.014–99%) of the total auxin content. The ABA content varied from negligible levels in three species to 54 $\mu\text{mol}\cdot\text{kg}^{-1}$ (DW) and typically significantly decreased after overwintering. Among all functional traits examined, hormone profiles most strongly depended on the turion sprouting position (surface/bottom), indicating that this trait is critical in turion ecophysiology. The contents of several ABA catabolites (PA, DPA, neoPA, 7'OHABA) decreased significantly after overwintering, whereas that of ABA-GE remained relatively unchanged. With some exceptions, our findings confirm the key role of ABA in regulating turion dormancy. However, the results indicate that the hormonal mechanisms underlying turion development and breaking dormancy in turions are not uniform within all aquatic plants.

INVESTIGATING THE ROLE OF PLANT FORMINS IN CLATHRIN-MEDIATED ENDOCYTOSIS

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Key words: clathrin, endocytosis, formins, plant

Class I formins in plants are integral membrane proteins that facilitate actin nucleation and contribute to cytoskeletal organization. Although these formins are localized to the plasma membrane, and proteomic analyses have identified their presence within clathrin-coated vesicles, implicating a potential role in clathrin-mediated endocytosis (see F. Cvrčková et al., 2024). Preliminary experimental evidence supports this hypothesis. Pharmacological inhibition of formin activity using SMIFH2 markedly reduced the lifetime, velocity, and density of GFP-tagged clathrin-coated vesicles in *Arabidopsis* root epidermal cells. Furthermore, colocalization analyses employing p35S:CLC:GFP and pFH1:FH1:mScarlet demonstrated transient colocalization between AtFH1 and CLC:GFP-labelled vesicular structures, which subsequently underwent dissociation. Collectively, these findings indicate a mechanistic involvement of formins in clathrin mediated endocytosis. Elucidating their precise role is likely to advance our understanding of the interplay between membrane trafficking and cytoskeletal dynamics in plant cells.

Acknowledgement

Thanks to the TANGENC reg. no. CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes Amos Comenius.

DROUGHT IS A CRUCIAL FACTOR AFFECTING PRODUCTION OF QUERCETIN-GLYCOSIDES IN LOTUS SP.

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Key words: drought, flavonol, glycosides, quercetine, stress metabolites,



Flavonoids, especially quercetin and its glycosides, are widely occurring plant stress metabolites that play an essential role in defence against oxidative stress, interaction with plant stress response signalling, and regulation of plant growth and development. The content of their compounds in plants is dependent on several environmental factors. *Lotus* sp. produce considerable amounts of quercetin glycosides; however, their level in plants grown in controlled and stable conditions is generally low. The present work focused on the factors that could play a crucial role in the induction of quercetin glycosides production, and drought was found to be the strongest factor. During drought, the total amount of quercetin glycosides increased more than 5 times, and the expression of enzymes of quercetin biosynthesis (flavanone 3 β -hydroxylase, flavonol synthase and flavonoid 3'-hydroxylase) was strongly induced. In parallel, a considerable increase in expression of several genes for UDP-glucosyltransferases was also detected, especially those of LjUGT78K9 and LjUGT73C17, which are likely involved in flavonol glycolysation. The accumulation of isoflavonoids, which is also induced by different stress conditions, was affected to a lesser extent by drought.

Acknowledgement

Authors acknowledge the financial support of the project VEGA 1/0452/24 from the Ministry of education, science, research and sport of the Slovak Republic.

PRODUCTION OF ANTHRAQUINONES IN ENDOPHYTIC FUNGUS *TALAROMYCES ISLANDICUS*

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Keywords: anthraquinones, endophyte, *Talaromyces*

The polyketide-derived fungal anthraquinones (AQs) share emodin-like structure or are dimers of AQ monomers yielding bisanthraquinones with known anti-inflammatory, bacteriostatic and antitumor activities. *Talaromyces islandicus* (Taxonomy ID: 28573), an endophytic fungus of *Laurencia okamurai*, produces more than 20 different AQs, including monomeric (emodin, chrysophanol, islandicin, endocrocin, catenarin), dimeric (skyrin, oxyskyrin, isoskyrin) and modified bisanthraquinones with cage structure (rugulosin, rubroskyrin, luteoskyrin). We evaluated *T. islandicus* production of AQs using six types of commercially available solid media. The effect of carbon source, glucose or sucrose, was also tested. Small-scale extraction of metabolites was carried out with a modified version of the plug extraction method and analyzed by HPLC. *T. islandicus* growing on PDA (Potato dextrose agar), CZA (Czapek-Dox agar) and MEA (Malt extract agar) produced skyrin and skyrin hexoside as its main AQs. The highest amount of skyrin was detected on CZA on the 21st day of culture reaching 20-fold higher concentration compared to other media. MSA (Murashige and Skoog medium) and SDA (Sabouraud dextrose medium) stimulated production of monomeric AQs, mainly islandicin, emodin and its glycosides. Extracts from SOY (Soyabean casein digest medium) contained only traces of AQs. As the used media differed in carbon source, PDA and SDA contain 2% glucose, and MSA and CZA contain 3% sucrose, we tested effects of these concentrations. Glucose-enriched media stimulated higher production of AQs, including emodin, chrysophanol, islandicin and skyrin, than sucrose-based media, but more types of AQs derivatives were detected on commercially available media. The ability of *T. islandicus* to stably accumulate several AQs, including host plant-specific, combined with targeted elicitation of preferred subset of AQs can serve as a model for a biotechnological production and OMICs studies aim at elucidation of AQ biosynthesis and regulation in plants.

Acknowledgement

This work was supported by the Scientific Grant Agency VEGA 1/0546/22 and the Cultural and Educational Grant Agency KEGA 015UPJŠ-4/2024. Special thanks go to prof. RNDr. Eva Čellárová, DrSc.

ANALYSIS OF VOLATILE SIGNATURES ASSOCIATED WITH LATE BLIGHT DISEASE

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Key words: GC-MS, markers, *Phytophthora infestans*, VOC

Plants release volatile organic compounds when interacting with pathogens. Among the major pathogens is *Phytophthora infestans*, which poses a serious threat to agricultural production. There is still limited information about the volatile substances that plants release in response to a *P. infestans* infection or those produced by the pathogen itself, even though they have been studied previously. The study focused on testing various static and dynamic headspace techniques and employed advanced high-resolution mass spectrometry coupled with gas chromatography to identify and quantify volatile organic compounds produced by pathogen and plants. Utilizing an advanced analytical approach, we identified new cyclic alkanes not described in previous studies, which are promising markers for detecting the presence of pathogen. Furthermore, analysis of plant species with varying resistance to *P. infestans* from the *Solanaceae* family revealed that they produce completely different spectra of substances that may play a role in resisting *P. infestans*. Infected potato plants showed a different response in the accumulation of monoterpenes and sesquiterpenes, along with a specific increase in aldehydes that were not detected in control plants. Therefore, the work yielded promising results that can be utilized in future studies or applied research for new detection techniques.

EXPLORING THE BIOTECHNOLOGICAL POTENTIAL OF MOSSES

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Key words: biotechnology, heterologous expression, mosses, recombinant proteins

The search for efficient and cost-effective eukaryotic expression systems for production of recombinant or industrially important proteins is one of the main goals in the field of biotechnology. Mosses represent an intriguing platform for heterologous protein expression due to their low level of structural complexity and predominant haploid gametophytic growth, high rate of homologous recombination and efficient transformation. Furthermore, substantial biomass production during the gametophytic growth under in vitro conditions is essential for achieving high yields of recombinant proteins. We established and optimized axenic moss cultures on solid and liquid media, PEG-mediated transformation, antibiotic selection and regeneration of transformants. We successfully performed transient and stable expression of simple (turboGFP and human antimicrobial protein) and more complex (calpain domain of DEK1 and stearyl-CoA desaturase) proteins in mosses using either constitutive (*ubiquitin*) or inducible (*heat-shock*, *cold-shock*) eukaryotic promoters. Target proteins were fused with turbo GFP and analysed by confocal and fluorescence microscopy. Preliminary results demonstrate enhanced growth and increased fresh biomass production of filamentous protonemal tissue. Strong and stable fluorescent signal of selected recombinant fusion proteins were observed in the cytosol, nucleus, and endoplasmic reticulum of protoplasts or protonemal cells, depending on the protein type. These findings highlight the potential of mosses as an alternative and sustainable platform for heterologous protein expression in biotechnology.

PROXIMITY LABELLING OF THE CYCLIC NUCLEOTIDE GATED CHANNELS INDICATES NEW SIGNALING PATHWAYS TRIGGERING PLANT CALCIUM SIGNALING

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Key words: CNGC, Calcium, proximity labelling

Cyclic Nucleotide-Gated Channels (CNGCs) represent the largest group of calcium channels in plants. They are activated by a wide range of stimuli, including auxin, abscisic acid, heat, cold, pathogen-associated molecular patterns, and more. Recently, we demonstrated that all land plant CNGCs require additional proteins—Armadillo Repeat Only (ARO) proteins—for their proper function. AROs contain two sets of armadillo repeats, which are common protein-protein interaction scaffolds. This raises the question: can AROs integrate CNGCs into a larger molecular complex, or “channelosome”?

To investigate this, we generated a comparative proximiome of different CNGC channels in wild-type and ARO-deficient (*aro2/3/4*) plants to identify ARO-dependent CNGC interactors. Our analysis confirmed the presence of all three sporophytic AROs within the CNGC proximiome. Additionally, we identified several novel interactors that not only support previously known CNGC heterotetramerization but also suggest CNGC involvement in new signaling pathways.

Acknowledgement

This project was supported by the Czech Science Foundation grant Nr. 25-16449S and by European Union, Horizon Europe, project MOLIPeC, ID 101087030.

EFFECT OF ATFH5 FORMIN MUTATION ON ACTIN DYNAMICS AND STABILITY UNDER SALT AND OSMOTIC STRESS IN *ARABIDOPSIS THALIANA*

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Key words: actin dynamics, AT5G54650, kymogram, salt stress, structure stability

The plant cytoskeleton is a dynamic network of protein filaments, including microtubules and actin filaments. Actin and microtubule organization is modulated by a range of proteins, including formins. The *Arabidopsis* Formin Homologue 5 (AtFH5: AT5G54650) is a class 1 formin capable of nucleating actin assembly. It also contributes to seedling salt tolerance since loss-of-function *atfh5* mutants exhibited increased sensitivity to NaCl stress. In this study, fluorescently tagged cytoskeletal markers were used to track actin dynamics and stability under salt stress in wild-type and *atfh5* mutant plants using confocal spinning-disk microscopy. Quantitative Analysis of Cytoskeletal Kymograms (QUACK) were applied to quantify actin dynamics and lifetime structure. Hence, treatment with 150mM NaCl significantly reduced actin dynamics and disrupted actin structural stability in *fh5* mutant plants. Additionally, the application of 10mM CaCl₂ also significantly decreased actin dynamics in *fh5* mutants compared to wild-type plants. However, both *fh5* and wild-type plants showed similar disruption in Lifeact-GFP signal stability under CaCl₂ treatment. Furthermore, high concentrations of mannitol were found to disturb the plant cytoskeleton. Treatment with 300mM mannitol significantly reduced actin lateral mobility in both genotypes, with a more pronounced effect observed in *fh5* mutants. While short term treatment with 0.1μM Latrunculin B alone increased sensitivity to salt stress, co-treatment with LatB and 150mM NaCl did not show an additive effect on cytoskeletal behaviour. These

findings suggest that beyond its role in actin nucleation, AtFH5 contributes to the regulation of actin dynamics and structural stability under salt stress conditions *in vivo*.

Acknowledgement

The work was supported from the project TowArds Next GENeration Crops, reg. no. CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes Amos Comenius.

BEYOND HYPERICIN: METABOLIC INSIGHT INTO EMODIN- AND SKYRIN-TYPE ANTHRAQUINONES IN *HYPERICUM* SPECIES

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Keywords: emodin, glycosides, HPLC-DAD, LC-MS, oxyskyrin

Anthraquinones (AQs) represent the largest group of naturally occurring quinones, comprising over 700 compounds found in plants, fungi, lichens, and insects. Known for their antioxidant, anticancer, anti-inflammatory, and antimicrobial properties, AQs also contribute to the pharmacological profile of *Hypericum perforatum* L. (Hypericaceae), a model species studied primarily for hypericin and pseudohypericin. While emodin is the main AQ monomer in *Hypericum* spp., recent findings suggest the presence of a broader spectrum of emodin-derived metabolites.

This study investigates the interspecific variation in AQ composition across 11 *Hypericum* species with varying hypericin content to explore potential link between AQ profiles and naphthodianthrone biosynthesis. Extracts were analyzed using HPLC-DAD for qualitative and quantitative screening. Due to limited availability of reference standards, especially for glycosylated derivatives, LC-MS analysis was essential for structural characterization of the most abundant AQs.

Chromatographic profiles of hexane and ethyl acetate fractions revealed several distinct AQ peaks, including emodin-like monomers and skyrin-related dimers. The identified metabolites included emodin, its *O*-glucoside, and structurally related *O*-pentosides and hexosides of skyrin and oxyskyrin. Total AQ content ranged from 0.001 µg/mg DW in *H. maculatum* to 0.250 µg/mg DW in *H. humifusum*. Correspondingly, the lowest hypericins content, 2.100 µg/mg DW, was found in *H. maculatum*, while the highest amounts were present in *H. perforatum* and *H. humifusum*, 21.800 µg/mg DW and 24.800 µg/mg DW, respectively.

Our findings highlight substantial interspecific variability in both the spectrum and abundance of AQs in *Hypericum* spp. These differences may reflect metabolic branching points relevant to the biosynthesis of hypericins and suggest a potential role for specific AQs and their glycosides in the polyketide pathway.

Acknowledgement

This work was supported by APVV-18-0125, VEGA 1/0546/22, and KEGA 015UPJŠ-4/2024. The part of research was conducted in the laboratories of the Institute of Analytical Chemistry of the CAS (Institutional research plan RVO:68081715).

COMBINED EFFECT OF ELEVATED ATMOSPHERIC CO₂ CONCENTRATION AND NITROGEN AVAILABILITY ON THE METABOLISM AND PHYSIOLOGY OF *CALAMAGROSTIS VILLOSA*

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Key words: elevated atmospheric CO₂, nitrogen availability, secondary metabolism

Changes in atmospheric CO₂ concentration directly influence plant physiology and growth. To predict the responses of natural plant communities to future increases in CO₂, it is crucial to understand how species and ecosystems respond to elevated CO₂ and their ability to exploit newly created niches. While much of the research in forest ecosystems has focused on trees, understory communities received only limited attention, despite their essential role in maintaining species diversity, habitat stability, and ecosystem processes.

This study investigates the responses of *Calamagrostis villosa*, a grass species representative of understory vegetation, to elevated (EC, 700 ppm) and ambient (AC, 400 ppm) CO₂ concentrations, with varying nitrogen availability. The experiment was conducted in experimental lamellar domes at the Bílý Kříž site in the Beskydy Mountains. Photosynthetic characteristics were measured using an open gasometric system, while samples were collected for broad-spectrum metabolomics and elemental analyses.

Results showed that elevated CO₂ increased photosynthetic CO₂ assimilation rates, with N availability having no significant effect. However, reduced N availability increased the variability in CO₂ assimilation. Elevated CO₂ also enhanced water use efficiency, with slight stimulation observed under higher N availability. In contrast, N availability significantly reduced the C:N ratio, while elevated CO₂ had less of an effect. Phenolic acids, such as vanillic and syringic acid, generally decreased with higher N availability, whereas the effect of elevated CO₂ depended on N availability. Other phenolic compounds were slightly stimulated by elevated CO₂, with nitrogen availability having a bigger effect. These findings indicate the complex interaction effects of elevated CO₂ and nitrogen availability on plant physiology.

Acknowledgement

This research was financially supported by the Internal Grant Agency of MENDELU (AF-IGA2022-IP-044) and the AdAgriF project (CZ.02.01.01/00/22_008/0004635).

SPERMOSPHERE: THE COMPOSITION OF SEED EXUDATES SHAPES SEED – MICROBIOME INTERACTIONS

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Key words: microbiome, metabolites, pea, seed exudates, spermosphere

Seeds play a pivotal role in shaping the future of plants, influencing development at multiple levels. Seed exudate composition plays a crucial role in shaping seed-microbiome interactions, influencing both seed establishment and plant health and prosperity. The composition can either suppress the growth of pathogenic organisms or stimulate the proliferation of beneficial microbes. While the specific roles of many individual compounds remain poorly understood, elucidating their function is essential for advancing agricultural sustainability and scientific understanding.

In this study, we investigate the role of seed exudates and their constituent metabolites in modulating microbial interactions. Our experiments demonstrate that phenolic compounds, particularly salicylic and ferulic acid, exhibit strong inhibitory effects on the pathogenic fungi *Sclerotinia sclerotiorum*, *Alternaria alternata*, and *Fusarium oxysporum*. These findings underscore the potential of seed-derived phenolics as natural defense agents against seed- and soil-borne pathogens. Ongoing metabolic and proteomic profiling of exudates from contrasting pea genotypes is being conducted to explore the relationship between seed biochemical traits and associated microbial communities. Interestingly, while the growth of certain microbes is suppressed by exudate compounds, others are attracted and utilize them as carbon sources, highlighting the dual role of seed exudates in both defense and microbial recruitment. This duality suggests a dynamic and selective interaction within the spermosphere.

Our findings emphasize the complexity of seed-microbiome interactions and reveal the potential of manipulating seed exudate composition as a strategy for enhancing crop resilience and promoting soil health. A deeper understanding of these mechanisms may enable the development of more sustainable and microbiome-informed agricultural practices.

Acknowledgement

This study received the support from Czech Science Foundation (24- 10730S) and Palacký University Olomouc (IGA_PrF-2025-001) projects.

BIOCONTROL AND GROWTH PROMOTION BY IAA-PRODUCING RHIZOBACTERIA ISOLATED FROM TOMATO (*SOLANUM LYCOPERSICUM* L.) RHIZOSPHERE.

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Key words: biocontrol agents, indole-3-acetic acid (IAA), rhizosphere microbiome, systemic resistance (ISR)

This study investigated the potential of rhizosphere-associated bacteria from tomato (*Solanum lycopersicum* L.) to enhance plant growth and disease resistance through indole-3-acetic acid (IAA) production and other defense-related mechanisms. Bacterial isolates were screened for IAA biosynthesis and evaluated for their ability to induce systemic resistance (ISR) against phytopathogens. Selected strains exhibiting high IAA production were further characterized for biocontrol traits, including siderophore-mediated iron competition, antifungal activity, and phosphate solubilization. Molecular identification revealed several isolates belonging to *Pseudomonas* and *Bacillus* genera, known for their plant-protective capabilities. The most promising strains not only stimulated root architecture through phytohormone production but also demonstrated significant *in vitro* suppression of fungal pathogens. These findings suggest that native IAA-producing rhizobacteria can serve as dual-function bioagents, promoting tomato growth while enhancing inherent disease resistance—a valuable strategy for reducing chemical inputs in sustainable agriculture.

NEW PHYTOHORMONE DERIVATIVES AS A MODERN TOOL FOR BASIC AND APPLIED PLANT RESEARCH

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Key words: auxin, cytokinin, *in-vitro* micropropagation

Cytokinins (CKs), in concert with auxin, are critical for successful plant regeneration in tissue culture, where the most frequently used CK is 6-benzylaminopurine (BAP). However, due its fast *in-situ* N9-glucosylation, it can also generate diverse negative effects, which complicate micropropagation processes, especially in recalcitrant species. To solve this problem, N9-glucosylation could be suppressed by appropriate N9 purine substitution of BAP or hydroxylation of its benzyl ring. Series of CK derivatives substituted at N9-position by various sugars and protective groups have been recently prepared, including their isotopically and fluorescently labelled analogues as well as their water-soluble salts, to improve CK specific biological activity and are already routinely used in plant micropropagation. Moreover, by small change in cytokinin structure, a potent cytokinin antagonists and/or inhibitors of their inactivation have been obtained.

Phytohormones auxins, thanks to their capacity to determine plant architecture, also have been successfully employed to obtain more economically advantageous plants *in vitro*. The ratio between activity of auxin and cytokinin needs to be tightly controlled to achieve proper shoot generation as well as rooting and acclimation. Recently we prepared a set of new PEO-IAA-inspired anti-auxins capable of antagonizing auxin responses *in vivo* and successfully used them to facilitate hemp micropropagation. Here, recent results of synthesis, characterization and biological activity testing of several new phytohormone derivatives will be presented and demonstrated that they can be used as an interesting new tool for plant biotechnology and agriculture.

Acknowledgement

This research was financially supported from European Regional Development Fund – Project “SMART Plant Biotechnology for Sustainable Agriculture” (No. CZ.02.01.01/00/23_020/0008497).

APPLICATION OF TOPOLIN-TYPE CYTOKININS DURING THE PROLIFERATION OF NORWAY SPRUCE EMBRYOGENIC CULTURES

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Key words: image analysis, *Picea abies*, somatic embryogenesis, topolin-type cytokinins

The process of conifer somatic embryogenesis (SE) is controlled by phytohormones exogenously supplied into the culture medium. The early phases of SE (induction and proliferation), which significantly influence the later development of somatic embryos and germination, are controlled by auxins and cytokinins (CKs). In traditional protocols, N6-benzylaminopurine (BAP) is the dominant CK. However, BAP and its degradation products are stable, causing adverse effects in many *in vitro* plant propagation systems.

We applied aromatic topolin-type CKs meta-topolin (mT), methoxy meta-topolin (memT), and meta-topoline riboside (mTR) during the proliferation stage of Norway spruce SE and monitored the developmental process until the germination stage. The effects of different CKs on the anatomical structure (specifically the area of meristematic centres) and the overall growth of proliferating cultures were assessed. The yield of high-quality mature embryos was recorded, and the percentage of germinating embryos, along with morphological parameters of emblings (including root and hypocotyl length), were monitored across various types of pre-germination treatments. Despite high genotype-dependent variability, the area of meristematic centres and yield of mature embryos were explicitly affected by the applied CKs. The germination parameters, however, were more dependent on the type of pre-germination treatment than on the CK applied.

Acknowledgement

The work was supported by the INTER-COST-LUC24 project (No. LUC24054) of the Ministry of Education, Youth and Sport of the Czech Republic (COST Action CopyTree) and European Regional Development Fund-Project “SMART Plant Biotechnology for Sustainable Agriculture” (No. CZ.02.01.01/00/23_020/0008497).

DIFFERENTIAL RESPONSES OF SELECTED POPLAR VARIETIES ON ARSENIC STRESS

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Key words: arsenic, phytoremediation, poplar, stress marker

Plants exposed to abiotic stressors such as heavy metals and metalloids face severe physiological disruptions, particularly under arsenic (As) and antimony (Sb) toxicity. These elements trigger overproduction of reactive oxygen and nitrogen species, leading to lipid peroxidation (increased malondialdehyde, MDA) and marked declines in chlorophyll content. To mitigate oxidative damage, plants upregulate antioxidant enzymes (such as superoxide dismutase, SOD, or peroxidase, POX) and accumulate osmoprotectants like proline, while modulating photosynthetic pigments and membrane integrity. Poplar species, recognized for rapid growth and phytoremediation potential, provide a useful model for studying metal and metalloid tolerance and stress responses, yet effects of As on biochemical stress markers in poplar varieties remain insufficiently understood. Our study addresses this gap by profiling antioxidant activity, proline, and MDA responses in five diverse poplar varieties under As stress. Leaves from stressed explants were frozen, and extracts from grind tissues used for spectrophotometric determination of antioxidant enzyme activity, proline, and MDA contents. Differential responses of the varieties will hopefully allow us to identify candidates capable of growing on contaminated soils without any major problems.

Acknowledgement

This work was supported by Slovak Grant Agency VEGA, grant 2/0047/25 and Slovak Research and Development Agency, grant APVV-23-0318. We thank for the technical support to Irena Mravíková.

RESPONSES OF APOPLASTIC BARRIERS TO ESSENTIAL NUTRIENT DEFICIENCIES

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Key words: exodermis, nutrient stress, split-root, adaptation

Our study focuses on the exodermis, a protective apoplastic barrier of root outer cortex, and analyses its response to deficiency of selected essential nutrients (N, P, K, Fe). We investigate whether the change in exodermal maturation rate under nutrient stress is a generally common trait among agronomically important crops and whether it differs between monocot and dicot species. We grow plants in hydroponic culture using homogeneous and split-root setup (to test for the localized response). The presence of differentiated exodermis was detected by histochemical staining of hand-microtome sections. Suberin lamellae were stained with Sudan Red 7B and lignified Casparian bands with berberine hemisulphate and crystal violet. The results show that all the species tested (*Sorghum bicolor*, *Triticum aestivum*, *Hordeum vulgare*, *Allium cepa*, *Helianthus annuus*, *Solanum lycopersicum*, *Linum usitatissimum*, *Capsicum annuum*, and model species *Brachypodium distachyon*), representing both monocots and dicots, formed exodermis, although the onset of its differentiation along the root axis varied among species. Nitrogen deficiency had the most significant and consistent stimulatory effect on exodermal maturation. Its magnitude varied among species, but with no consistent difference between monocots and dicots. *T. aestivum* and *H. vulgare* formed exodermis very close to the root base and responded mildly to deficiency, mild response was also found in *L. usitatissimum* with exodermal differentiation very close to the root tip. All other species formed exodermis significantly faster in N-deficient conditions. We also detected locally enhanced formation of exodermal Casparian bands in N deficient roots in split-root culture in *H. annuus*, *C. annuum* and *B. distachyon*. These results highlight that the exodermal response to nutrient stress is a common trait, with some species-specific differences suggesting that further research should focus on individual species to understand root system functions and adaptations to nutrient stress.

Acknowledgement

GAUK No. 336122

TowArds Next GENeration Crops, reg. no. CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes Amos Comenius.

CAN SILICON SUPPLEMENTATION STRENGTHEN SORGHUM'S DEFENSE AGAINST APHIDS? INSIGHTS INTO MORPHOLOGICAL AND BIOCHEMICAL RESPONSES

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Key words: aphids, oxidative stress, plant defence, silicon, *Sorghum bicolor*

Aphids are among the most destructive pests that affect agricultural crops worldwide, with chemical insecticides remaining the primary method of control. Due to the environmental risks of insecticides, such as soil degradation and harm to beneficial insects, sustainable alternatives are needed. Silicon shows promise in improving plant resistance to various stresses. This study aimed to evaluate the efficacy of silicon supplementation in enhancing the defence mechanisms of *Sorghum bicolor* against aphid infestation. The plants were cultivated in a silicon-enriched soil substrate and subjected to controlled aphid exposure. After the cultivation morphological and production parameters were assessed to calculate tolerance indices. To investigate the biochemical response, we quantified superoxide radicals and measured the activity of superoxide dismutase, a key antioxidant enzyme involved in mitigating oxidative stress. While silicon at the tested concentration did not markedly enhance tolerance to aphid infestation, the morphological and physiological responses, especially observable in the younger shoot organs, suggest substantial potential. These findings support further exploration of silicon in sustainable pest management strategies.

Acknowledgement

The project was supported by VEGA1/0745/20, UK/360/2023 and UK/3197/2024 grants.

A PROTEO-TRANSCRIPTOMIC APPROACH TO CHARACTERIZE THE HEAT STRESS RESPONSE OF *ARABIDOPSIS THALIANA* SEEDS

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Key words: alternative splicing, *Arabidopsis thaliana*, heat stress, multiomics, seed

Here, I present how we used proteomics and transcriptomics methods to decipher the heat stress response of *Arabidopsis thaliana* seeds at two different developmental stages—two days after pollination and three days after pollination. We examined differential expression to identify genes whose protein and transcript abundances changed, followed by calculating the correlation between changes in protein and transcript levels after heat stress. Additionally, we analyzed genes that were alternatively spliced at the transcriptomic level and searched for peptides that would confirm the translation of these transcript isoforms at the proteomic level.

The results show that genes differentially expressed ($\log_2FC > 1$ and adj. P. val < 0.05) at both the proteomic and transcriptomic levels are in the minority. Most differentially expressed genes were found to be regulated at either the proteomic or the transcriptomic level, but not both. Despite this, our data show a strong correlation—greater than 0.7—between proteomic and transcriptomic changes. We also demonstrated that a few peptides were detected at the proteomic level, supporting the conclusion that alternative splicing has a measurable impact on protein abundance. The Gene Ontology terms enriched after heat treatment were similar at both transcriptomic and proteomic levels, including terms such as heat stress response and heat stress tolerance. However, the terms enriched among alternatively spliced genes were distinct and did not include heat stress response or tolerance. This suggests that alternative splicing and heat stress are both important for the heat stress

response but affect different sets of genes involved in plant growth and development. Furthermore, we observed that even a small developmental difference—just 24 hours between the two stages—resulted in significant variation in differentially expressed and spliced genes. This indicates that the heat stress response differs notably between these two developmental stages in the seed.

Acknowledgement

Unravelling the involvement of PRP8 in mRNA splicing during embryogenesis and in seed thermoresponse, MU code GA22-29717S.

LYS10: NOVEL PROBE CAPTURING PECTIN DYNAMICS IN PLANT CELL WALLS

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Key words: cell wall, fluorescence imaging, homogalacturonan, peptide probe

The plant cell wall is a dynamic structure that undergoes constant remodeling to accommodate growth, differentiation, and responses to the environment. Pectic homogalacturonan (HG) is one of the key cell wall polysaccharides, which plays a crucial role in regulating cell wall mechanical properties and cell-to-cell adhesion. Despite its importance, tracking HG spatiotemporal dynamics *in vivo* remains challenging as traditional immunolabeling has been proven largely ineffective for such applications. To overcome these challenges, we developed and characterized a novel oligocationic peptide probe, a decamer of L-Lysine (Lys10). It is designed to associate with negatively charged regions of HG. Specificity analysis using a dot blot assay confirmed Lys10's binding to pectin isolates; the strength of the binding was relative to the level of methyl esterification. Using fluorescence microscopy, we demonstrated that Lys10 efficiently penetrates live plant tissues and labels cell walls throughout Arabidopsis root, making it suitable for live cell real-time imaging. This approach helped us to visualize HG's dynamic changes and distribution during root hair elongation and possible hot spots of pectin methylesterase activity. Furthermore, molecular docking revealed a high-affinity interaction between Lys10 and de-esterified homogalacturonan ($KD = 6,04 \times 10^{-9}$ M), comparable to antibody-based recognition. These findings demonstrate the potential of peptide-based probes for high-specificity cell wall imaging. Encouraged by Lys10's performance, we continue to develop additional probes targeting various polysaccharides, further expanding the tool repertoire available for studying cell wall remodeling during plant development.

Acknowledgement

Funded by NextGenerationEU through the Recovery and Resilience Plan for Slovakia under projects No. 09I03-03-V02-00005 and 09I01-03-V05-00010, grant of Slovak Academy of Sciences IM-2021-23 and VEGA 2/0162/24.

PARAPHAEOSPHAERIA NEGLECTA: AN UNNEGLECTED PRODUCER OF LACCASES AMONG HYPERICUM-BORNE ENDOPHYTES

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Key words: endophytic fungi, *Hypericum*, laccase activity

Endophytic fungi occur naturally in plant tissues and may produce bioactive compounds specific to host, albeit in small amounts. Some endophytes use laccases, a group of multicopper oxidoreductases, to degrade lignin and defend against stress factors. These enzymes are able to oxidize a wide variety of phenolic and non-phenolic compounds. They are candidates for the dimerization reaction leading to hypericin, a potential antitumor drug, produced in the plant kingdom exclusively by some members of the genus *Hypericum*. Therefore, obtaining endophytic isolates from *Hypericum* spp. with high laccase activity might contribute to elucidation of key biosynthetic steps.

The aim of this work was to identify endophytes from *Hypericum* representatives based on the ability to produce laccases. To achieve this aim, *in vitro* cultures of 21 *Hypericum* species were acclimated to outdoors. Altogether 283 samples were isolated from roots, stems and leaves over two years. Production of laccase was confirmed by 1-naphtol screening in 29,3% of endophytes. Laccase activity was significantly associated with sampled organs ($p=0.002$) and overwintering of plants ($p=0.022$). Relative risk analysis further revealed that fungi from leaves or overwintered plants had significantly higher laccase activity, while root-associated fungi had significantly lower activity. Fungi showing positive reaction were identified by ITS rDNA marker. The most abundant endophytes were *Alternaria alternata* - 14 isolates, *Diploceras hypericinum* - 12 isolates and *Fusarium* sp. - 4 isolates. Finally, production of laccase was quantified using 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonate) as a substrate in medium supernatant after 12 days of culture in potato dextrose broth. The highest production of extracellular laccases was observed for *Paraphaeosphaeria neglecta* isolate with mean production of 62.1 U/l. These findings contribute to the knowledge on distribution of plant colonizing endophytes with significant laccase activity.

Acknowledgement

This work was supported by VEGA 1/0546/22, KEGA 015UPJŠ-4/2024 and APVV-18-0125.

CHANGES IN GRAPEVINE PHYSIOLOGICAL PARAMETERS CAUSED BY WATER DEFICIT

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Key words: fluorescence; gas exchange; grapevine; water stress

The impact of water deficit affects crop production worldwide, including grapevine. Vine cuttings were grown in 5x5 cm containers in sterile perlite. The temperature regime in the greenhouse was 25 °/20 °C, humidity 70 ± 10 %. The light regime was natural. A greenhouse experiment was carried out on cuttings of Riesling, Tramin red and Pinot Noir grape varieties. The experimental scheme included 5 irrigation treatments: 120 ml (control) and 90 ml, 60 ml, 15 and 30 ml. Gas exchange rates were measured at weekly intervals. The results show that the Tramin and Riesling varieties show a similar trend compared to the Pinot Noir variety in the change in photosynthetic rate as a function of the duration of water deficit. Thus, there is a significant decrease in photosynthesis in the 15 ml variant. The transpiration rate also decreased as a function of the water deficit. Among the grapevine varieties studied, the Riesling variety showed the lowest transpiration rate compared to the Tramin and Pinot varieties. The fluorescence parameters were also affected by the experimental variant, with stressed plants showing the lowest values of Fv/Fm ratio. No differences were found between varieties. In conclusion, of the varieties studied, Tramin was the most sensitive to water deficit, while Pinot Noir appeared to be tolerant.

Acknowledgement

This work was created with the support of the grant project of the Ministry of Agriculture of the Czech Republic - NAZV QK21010189: Implementation of ecosystem services with a focus on water balance in viticultural practice.

SUSPENSION ESTABLISHMENT AND HEAT SHOCK RESPONSE OF GOLDEN GARDENIA (*GARDENIA SOOTEPENSIS HUTCH.*)

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Key words: *Gardenia sootepensis* Hutch., Plant tissue culture, Cell suspension culture, Heat stress response

Gardenia sootepensis Hutch., known as golden gardenia, is a medicinal evergreen tree native to Southeast Asia with notable antimicrobial, anti-inflammatory, cytotoxic, and antioxidant properties. Despite its pharmacological value, little is known about its cellular responses to abiotic stress, particularly heat stress. This study aimed to develop in vitro culture protocols and investigate heat tolerance in suspension cells derived from leaf explants. Callus induction was successfully achieved using Murashige and Skoog (MS) medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D; 0.5–2.0 mg/L) and kinetin (Kn; 0.1 or 0.2 mg/L), yielding a 100% induction rate. Calli formed with 0.5–1.0 mg/L 2,4-D were friable and olive-green, while 2.0 mg/L produced browning. Suspension cultures established with 0.5–1.0 mg/L 2,4-D and 0.1 mg/L Kn showed enhanced cell proliferation and biomass accumulation. Heat shock experiments revealed that *G. sootepensis* suspension cells tolerated exposure to 55°C (extracellular medium temperature of 46.7 ± 0.10°C) for 5 minutes without visible structural damage. These results demonstrate the species' potential for in vitro propagation and its initial resilience to heat stress. Further research is needed to refine culture conditions, assess long-term stability and genetic fidelity, and deepen understanding of stress response mechanisms.

Acknowledgement

This research was supported by University of Phayao, and Demonstration School, University of Phayao.

EFFECT OF ECOPHYSIOLOGICAL PARAMETERS AND SUSTAINABLE AGRICULTURAL PRACTICES ON ESSENTIAL OIL AND AGRONOMIC PERFORMANCE OF GREEK OREGANO

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Key words: biostimulants, carvacrol, essential oil, greek oregano, harvesting stage

Greek oregano (*Origanum vulgare* subsp. *hirtum*) is the most commercially significant oregano species and among the most widely cultivated aromatic and medicinal plants in Greece. It is utilized extensively as a culinary herb and for its essential oil (EO), whose principal constituents—carvacrol and thymol—are primarily responsible for its diverse bioactivities, including antimicrobial, antioxidant, antibacterial, and antifungal effects. The yield and quality of EO, as well as overall crop productivity, are influenced by multiple factors, including genotype, edaphoclimatic conditions, harvest timing, and agronomic practices. In this context, biostimulants have emerged as a sustainable agronomic tool, promoting plant growth, enhancing nutrient uptake efficiency,

and potentially reducing the dependence on synthetic fertilizers. Although substantial research has focused on the chemical variability of EO—mainly in wild oregano populations—limited data exist on the interactions between plant developmental parameters (e.g., flowering stage at harvest), local environmental conditions, and cultivation inputs such as biostimulants under systematic cultivation. The present study investigated the combined effects of soil-climatic parameters and physiological stages—specifically flowering stage—on EO yield and composition across multiple cultivation sites. Furthermore, it evaluated the impact of different biostimulant treatments on the agronomic performance of cultivated oregano. The results demonstrated significant regional variability in all examined parameters. EO yields ranged from 3.90% to 5.30% across locations and from 3.70% to 5.53% between the two harvest stages. Carvacrol concentration varied in relation to both environmental and phenological conditions. Biostimulant applications exhibited positive effects on crop performance, suggesting their potential as a sustainable alternative to conventional fertilization strategies.

Acknowledgments

This study is funded under the "Measure 16, in the framework of National Rural Development Programme and is co-financed by the European Union (EAFRD). Sub Measure 16.1 – 16.3, Project code M16SYN2-00057.

PLANT AND THE BEAST: HOST PLANT CHEMICAL RESPONSE TO OVIPOSITION BY DAMSELFLY (*LESTES*)

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Key words: cytokinins, oviposition, plant defense, stress phytohormones, UHPLC-MS/MS

Plants respond to oviposition by herbivorous insects by triggering a signal cascade similar to that which is well-known following injury to plant tissue. The eggs of herbivorous insects, as immobile and seemingly inactive stages, have been intensively studied over the last three decades when considering plant-herbivore interactions. However, it is still not known how a plant responds to oviposition by an animal that does not potentially threaten it but instead helps it figuratively by consuming herbivorous insects. Dragonflies are powerful predators that consume hundreds of thousands of those insects. From an evolutionary point of view, the question arises as to whether the plant adapts its response to the oviposition of an insect predator such as a dragonfly, potentially protecting the plant from insects living in the plant tissue. Our preliminary case study was performed to verify methods and it proved that monitoring of hormonal response is feasible and repeatable. The study analyzed the phytohormonal response of the plant common rush (*Juncus effusus*) to oviposition by the damselfly (*Lestes sponsa*) through quantitative analysis of stress phytohormones and cytokinins by ultra-high-performance liquid chromatography-tandem mass spectrometry. However, individual hormones and their crosstalk need more molecular and genetic data for a better understanding of the precise involvement of these pathways.

"WHO TRIGGERS WHO" IN EARLY RESPONSES OF PLANT PATTERN-TRIGGERED IMMUNITY

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Key words: Arabidopsis, M(D)-AMPs, ROS, Ca²⁺, CNG Channels

Plants recognize danger signals from damage or pathogens through pattern recognition receptors, which trigger rapid kinase activation and a surge of calcium. While different stimuli activate specific kinases, calcium responses appear uniform, leaving the balance between kinase-specific and general calcium-mediated effects unclear.

We recently identified *aro2/3/4* mutants with abolished calcium signaling across the entire CNGC (cyclic nucleotide-gated channel) family. We are systematically analyzing Ca²⁺ dynamics and reactive oxygen species (ROS) production, another early immune response, in *aro2/3/4* mutant plants in roots and shoots after treatment with broad set of microbe-associated molecular patterns (MAMPs) and damage-associated molecular patterns (DAMPs). We aim to identify the specific CNGCs responsible for mediating signaling to various danger signals and how the signal patterning differs in response to distinct MAMPs and DAMPs.

COMPARISON OF GENE PREDICTION MODELS FOR THE DISCOVERY OF CANDIDATE HYPERICIN BIOSYNTHETIC GENES IN *HYPERICUM* GENOMES

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Key words: comparative genomics, gene prediction model, in silico, *Hypericum*,

Representatives of the genus *Hypericum* are valuable producers of biologically active secondary metabolites, especially hypericin with anticancer activities. Hypericin is known to be biosynthesized via the polyketide pathway, but the exact steps remain unvalidated. It is assumed that two emodin molecules are coupled into dimerized anthraquinone precursor. This step could be catalyzed by cytochrome P450 or multicopper oxidase with laccase activity.

The objective of our work was to find the most accurate gene prediction model for the *Hypericum* spp genomes. We used two species contrasting with contrasting hypericin production abilities – *Hypericum perforatum* and *H. kalmianum* – along with public reference genomes for *H. androsaemum*, *H. hirsutum* and *H. pulchrum*. Gene prediction was performed with AUGUSTUS using models from eight dicotyledonous species. Annotation quality was assessed via BUSCO, and functional characterization of predicted proteins was conducted using eggNOG-mapper.

Among the tested models, *Ricinus communis*, *Vitis vinifera*, *Populus trichocarpa* and *Arabidopsis thaliana* were preferred based on the high number of predicted genes, complete BUSCOs, and reasonable proportion of ortholog matches. On average, the models predicted 62 multicopper oxidase genes and 482 cytochromes P450.

These findings underscore the value of model selection in genome annotation and provide a genomic foundation for elucidating the biosynthetic pathway of hypericin.

Acknowledgement

This work was supported by the Scientific Grant Agency VEGA 1/0546/22 and the Cultural and Educational Grant Agency KEGA 015UP-JŠ-4/2024 of the Ministry of Education, Youth and Sports of the Slovak Republic.

EFFECT OF CADMIUM AND UV-B IRRADIATION ON ENDOPOLYPLOIDIZATION OF *RAPHANUS SATIVUS* 'LADA'

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Key words: abiotic stress, cadmium, endopolyploidy, *Raphanus*.

One of the most serious ecological problems currently is pollution of soil by heavy metals. Cadmium is a metal that occurs in trace amounts in soil and water, where the sources of pollution are either geological or anthropological activity. Cadmium has toxic effects and slows down the cell cycle. With climate change, plants are also affected by excessive UV-B radiation, which damages DNA and photosystems. These abiotic stressors may reduce crop production. One of the mechanisms of stress tolerance and adaptation to stress is endopolyploidization. Endopolyploidization represents the multiplication of DNA content in the nucleus without subsequent mitosis, thereby increasing the ploidy level of the cell. We studied the effect of abiotic stress caused by two concentrations of cadmium and UV-B radiation on *Raphanus sativus* 'Lada'. Hydroponically grown plants were exposed to 15 μ M and 30 μ M cadmium and were subsequently irradiated with UV-B radiation. Endopolyploidy was measured using flow cytometry in hypocotyl tubers, petioles and leaf blades. The fresh weight of roots stressed by cadmium, UV-B and a combination of these stressors had decreased significantly. Only a slight increase in root biomass was observed at 15 μ M cadmium concentration. Generally, cadmium, UV-B and their interaction caused an increase of endopolyploidy levels in petioles and leaves, and 15 μ M cadmium caused an increase of EI in tubers. On the contrary, ploidy levels in tubers slightly decreased after being exposed to higher concentrations of cadmium, UV-B and the interaction of these two factors.

EVALUATION OF THE GENOME SIZE OF SELECTED MOSS SPECIES

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Key words: flow cytometry, genome size, mosses, C-value.

Genome size (C-value) represents the relatively constant DNA content in holoploid cell, i.e. cell with unreplicated genome, specific for given species. It is well known that this parameter influences several aspects in the life cycle, biology and ecology of plants – the duration of the cell cycle and cell division, the consequential cell size, and also the ability of plants to adapt to the habitat and tolerate stress. Mosses are a poorly studied group within the plant kingdom in terms of genome size. Currently, the estimates of genome sizes for approximately 250 species are known, representing less than 2% of all known mosses. We analysed axenic cultures of 27 moss species, for which we estimated genome size using flow cytometry. In studied mosses, the variability of genome size was relatively small, C values ranged from 0,19 to 2,21 pg. The smallest genome size among the tested species had *Anemodon rostratus* (0,19pg). Mosses with the largest genomes were *Plagiomnium rostratum* and *Pohlia cruda* with 2,1 and 2,21 pg respectively.

PHYTOREMEDIATION POTENTIAL AND BIOMASS PRODUCTION IN POPLAR CLONES EXPOSED TO ARSENIC AND ANTIMONY

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Key words: antimony, arsenic, biomass production, phytoremediation, poplar clones

Soil contamination with arsenic (As) and antimony (Sb) resulting from historical mining activities remains a serious and persistent environmental issue in many regions, including former mining districts in Slovakia. These metalloids, often co-occurring in contaminated sites, pose risks to ecosystems and human health due to their toxicity and mobility in soil systems. Among various remediation strategies, phytoremediation using fast-growing tree species offers a sustainable, cost-effective, and environmentally friendly alternative. Poplars are widely recognized for their high biomass production, adaptability, and tolerance to abiotic stress. We assessed the responses of five poplar clones, cultivated hydroponically and exposed to defined concentrations of As and Sb. The following key parameters were analyzed: photosynthetic pigment content, growth characteristics, and levels of phenolic compounds as biochemical indicators of stress. Preliminary data on elemental composition in aboveground tissues are also being considered to better understand uptake capacity and translocation potential. The results revealed considerable interclonal variation in physiological and morphological responses to metalloid stress. These findings highlight the dual potential of selected poplar clones for both phytoremediation and renewable biomass production. Such genotypes may be suitable for the reclamation of As/Sb-contaminated sites and offer a sustainable biomass resource for energy or industrial applications.

Acknowledgement

This work was supported by Slovak Grant Agency VEGA, grant 2/0047/25 and Slovak Research and Development Agency, grant APVV-23-0318. We thank Irena Mravíková for the technical support.

ENDOPHYTIC MITIGATION OF METAL STRESS IN *ARABIDOPSIS*: A CASE OF *SPOROBOLOMYCES RUBERRIMUS*

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Key words: Bioprecipitation, endophytic yeasts, carotenoids, heavy metals, *Sporobolomyces ruberrimus*

Presented study explores the role of *Sporobolomyces ruberrimus*, an endophytic yeast isolated from *Arabidopsis arenosa*, in mitigating the harmful effects of metal contamination in soil, particularly focusing on iron (Fe) and zinc (Zn). The fungus appears to regulate plant metal homeostasis and inactivate metals through precipitation, reducing their bioavailability and harmful effects. This study demonstrates that *S. ruberrimus* can precipitate iron as mixed oxides, hydroxides, or phosphates in amorphous forms, similar to the iron plaque found in wetland plants, but with a more complex composition. Notably, the fungus also adsorbs Zn ions onto the precipitate, suggesting a mechanism by which metals other than Fe may be inactivated. The research further investigates the yeast's response to metal stress, focusing on carotenoid biosynthesis under elevated Fe and Zn concentrations. High Fe³⁺ levels (above 350 μM) inhibit yeast growth, likely due to oxidative stress, while Zn²⁺ enhances growth and stimulates the synthesis of torulene/torularodine, a carotenoid that could play a protective role in oxidative stress conditions. Chromatographic analyses confirm that Zn²⁺ increases torulene/torularodine concentration in *S. ruberrimus* biomass over twofold, while β-carotene levels remain stable. Moreover, metal analysis shows that *S. ruberrimus* accumulates Zn²⁺ and significantly immobilizes Fe³⁺, likely through chelating organic compounds. The findings provide new insights into the potential applications of *S. ruberrimus* in phytoremediation, particularly in soils contaminated with heavy metals. Additionally, the increased production of torulene/torularodine under metal stress suggests its possible industrial applications in the food, cosmetic, and pharmaceutical sectors.

Acknowledgement

The research was funded by NCN grants: Miniatura 2023/07/X/ST4/00710, OPUS 2021/43/B/NZ9/03034, 2017/27/B/NZ8/01199.

REGULATION OF THE *DBCHIT1-3* CHITINASE GENE IN *DROSERA BINATA*: INFLUENCE OF ORGAN TYPE, INDUCERS, AND NUTRIENT SIGNALS

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Key words: hydrolytic enzymes, prey digestion, glucose repression, organ-specific expression

Drosera binata, a carnivorous plant adapted to nutrient-poor environments, utilises specialised leaf structures to capture and digest insects. Among the hydrolytic enzymes involved in this process, chitinases are critical for degrading the chitinous exoskeletons of arthropod prey. This study focused on the expression and regulation of the *DbChit1-3* gene, which encodes a chitinase. We analysed its organ-specific expression and transcriptional response to various inducers and repressors in *in vitro*-grown, non-treated *D. binata* plants. Expression analysis revealed that *DbChit1-3* is predominantly transcribed in leaf blades, with significantly lower levels in petioles and stems, and negligible expression in roots and flowers. After 24-hour treatments with solid compounds, *DbChit1-3* transcription was up-regulated by chitin (4.6-fold), gelatin (3.9-fold), and, notably, pachyman, a β -1,3-glucan (5.7-fold). Cellulose had no effect, while sand and laminarin—a β -1,3-glucan with a lower degree of polymerization—significantly suppressed gene expression. Time-course analysis showed that *DbChit1-3* mRNA levels peaked 24 hours after pachyman application, then declined. Additionally, glucose and N-acetyl-D-glucosamine—monomers of pachyman and chitin, respectively—strongly repressed *DbChit1-3* transcription, with maximal suppression (to 21–31% of baseline) observed 5 hours post-application. Laminarin solution also repressed expression, likely due to glucose release *via* endogenous glucanases. Co-treatment with glucose and pachyman resulted in only partial gene induction (60% of basal expression), indicating that nutrient availability overrides substrate-induced expression. These findings demonstrate that *DbChit1-3* expression in *D. binata* is tightly regulated by both substrate structure and nutrient signals, suggesting a complex control system that coordinates digestive enzyme production in response to prey availability and nutritional status.

Acknowledgement

The authors would like to thank Anna Fábelová for *in vitro* plant care and technical assistance. This work was supported by the project APVV-23-0448 and VEGA 2/0021/24.

EFFECT OF SALICYLIC ACID ON *ARABIDOPSIS THALIANA* METABOLISM AND CUTICLE PROPERTIES

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Key words: immunity, photosynthesis, plant cuticle, respiration, salicylic acid

Plants have evolved a sophisticated interconnected defence system against pathogens, traditionally divided into passive mechanical and chemical defences, and active immunity. Plant immunity is triggered when the molecules associated with pathogens are recognised. An important player in plant immunity is the salicylic acid (SA) phytohormone and its signalling pathway. However, the components of passive defence are not as passive as one might think; they change, renew, and evolve during the life of the plant organs. Triggered immunity is energetically demanding and can affect the development of passive defence systems. In this work, we focus on the effect of endogenously modulated concentration of SA on plant metabolism, and the development and properties of plant mechanical barriers. We used a collection of *Arabidopsis thaliana*

mutants with modulated SA concentration. Using LI-COR, we measured their photosynthetic and respiratory activity. By two different methods: calcofluor white staining and water loss, we compared their cuticle permeability. Using GC-MS, we also measure composition of cuticular waxes and cutin matrix. The data obtained contribute to a better understanding of how SA inhibits plant growth and how active immunity interacts with passive defence mechanisms.

Acknowledgement

This work was supported by the GAJU grant 027/2023/P.

TEMPORAL DYNAMICS OF ANTIOXIDANT ENZYMES DURING EARLY DARK-GROWN DEVELOPMENT IN MAIZE

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Key words: antioxidant enzymes, circadian regulation, dark-grown development, maize seedlings, *Zea mays* L.

Circadian clocks are internal timekeeping mechanisms that enable organisms to anticipate and adapt to predictable daily environmental changes. In plants, these endogenous rhythms coordinate various physiological processes, including antioxidant defense. In this study, we investigated time-of-day-dependent changes in antioxidant enzyme activities and peroxidase gene expression in maize (*Zea mays* L.) seedlings during the earliest stages of development under continuous darkness. Seedlings were cultivated in Petri dishes and sampled on the 3rd, 4th, and 5th day after germination at regular intervals over a 24-hour cycle. Growth parameters and enzymatic activities of guaiacol peroxidase (G-POX), ascorbate peroxidase (APX), and catalase (CAT), along with total soluble phenol content, were quantified spectrophotometrically. Additionally, temporal expression patterns of selected peroxidase genes were assessed using real-time PCR. These findings provide insight into the temporal regulation of antioxidant systems in dark-grown maize seedlings and suggest that early developmental processes may be influenced by endogenous timekeeping mechanisms, even in the absence of external light cues.

Acknowledgement

I would like to express my sincere gratitude to my supervisor, Zuzana Lukačová, for her invaluable guidance, support, and encouragement throughout this research. This work was supported by the Comenius University grant no. UK/1166/2025 and by the Slovak Research and Development Agency under contract Nr. APVV-17-0164, and VEGA1/0472/22.

HOW IS MRNA PROCESSING AFFECTED IN THE ABSENCE OF AN AGO BINDING PROTEIN NERD?

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Key words: *Arabidopsis thaliana*, Argonaut proteins, CRISPR/Cas9, mRNA processing, NERD

Argonaut (AGO) proteins are key mediators of small RNAs functioning. AGO proteins are bound by unstructured AGO-hook domains of various proteins involved in RNA-dependent DNA methylation. One of such proteins containing the AGO-hook domain in *Arabidopsis thaliana* is NERD (Needed for RDR2-independent DNA methylation), which is a plant-specific protein involved in RNA-dependent DNA methylation. Our experiments aim to analyze the phenotype of NERD knockout mutant plants and effects of



its absence on mRNA processing in *Arabidopsis thaliana*. We aim to compare these results with data previously generated on another AGO-hook containing protein of our interest, the essential transcription elongation factor of RNA polymerase II, SPT6L (Suppressor of Ty insertion 6-like). Since there are clues that NERD, like SPT6L, is an interactor of the RNA polymerase II complex, we hope to discover how the mRNA processing is affected by the impairment of their AGO-hooks. To achieve our goals, we analyzed and compared published data. We also crossed and selected NERD knockout T-DNA mutant plants and managed to reveal a T-DNA insertion related genomic duplication and translocation in the mutant line. To cope with this abnormality, we formulated a strategy of preparing mutant plants expressing NERD without the AGO-hook domain, which we generated using CRISPR/Cas9 genome editing.

Acknowledgement

Funding: GAUK no. 170125.

THE POTENTIAL ROLE OF SHUNGITE IN MITIGATION OF DROUGHT STRESS IN MAIZE PLANTS

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Key words: drought, maize, shungite

Drought as a result of climate change is an important factor negatively affecting crop production. Currently it is essential to apply new practices in cultivation of agricultural plants like selecting suitable crop species in farming regions or adding some amendments to the soil to modify its physical properties for plant growth. One of the key and strategic crops is maize. It is used as a food, forage crop, and it is also used for the industrial applications. Additives applied to the soil can improve water retention of soil, reduce evaporation, enhance resilience of plants, and reduce oxidative stress. In our study, we tested various contents of Karelian shungite stone powder, which can be easily mixed into the soil for enhancing drought tolerance of maize plants. The results indicate that shungite as the low-cost natural carbon material (in content 60 grams per kilogram of soil) has the positive and protective effect on growth parameters and amount of photosynthetic pigments in leaves of *Zea mays* exposed to water stress in comparison to the control variant without added shungite. Higher content of the shungite does not have a statistically significant effect on the monitored parameters.

Acknowledgement

This work was supported by Slovak Grant Agency VEGA (grant number VEGA 1/0472/22) and by the Slovak Research and Development Agency (grant number APVV-17-0164).

FROM TREATMENT TO STORAGE: LASTING BENEFITS OF PLASMA APPLICATION ON PEA SEEDS

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Key words: DCSBD, long-term effect, pea, plasma, temperature

Plasma treatment is a promising tool for enhancing seed germination, viability, and microbial decontamination. While its immediate effects have been widely studied, less attention has been paid to the longevity of these benefits during seed storage – a crucial factor in field agricultural use. In this study, we investigated the persistence of plasma-induced improvements in *Pisum sativum* var. Saxon seeds over a six-month storage period. Seeds were treated using a Diffuse Coplanar Surface Barrier Discharge (DCSBD) plasma source operating at atmospheric pressure in ambient air. After treatment, seeds were stored either at 4 °C or at room temperature. Our findings indicate that the positive effects of plasma treatment on germination parameters may be retained throughout the storage period. This confirms that plasma-treated seeds can be stored over extended periods without compromising the benefits of the initial treatment, making plasma technology viable for practical agricultural applications.

Acknowledgement

This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-21-0147.

ENVIRONMENTAL AND GENETIC REGULATION OF ADVENTITIOUS ROOT FORMATION AND ROOT-SHOOT JUNCTION (COLLET) PLASTICITY

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Key words: adventitious root, exocyst, plant, root-shoot junction

Root development is a very critical phase in plant life that plays a notable role in stress tolerance, production, nutrient and water intake. The differentiation of the root system and the root-shoot junction also known as collet, a transition zone which is regulated by intrinsic developmental processes and extrinsic environmental conditions. Adventitious root (AR) development is a critical feature reflecting this flexibility, but its control, particularly under different environmental conditions and genetic backgrounds, is not fully understood. This study explores how AR develops in *Nicotiana benthamiana* and *Arabidopsis thaliana* under various growing conditions. While *Nicotiana benthamiana* showed reduced AR development during shorter times but considerable increases with longer cultivation, showing species-specific responses, *A.thaliana* showed greater AR numbers under 5 days of dark treatment, and started decreasing by increased number of dark days treatment. Auxin signaling mutants in *Arabidopsis* (*tmk1-1*, *aux1*, *afb1-3*, *tir1-1*) showed decreased AR formation, demonstrating auxin's important role in RSJ and AR patterning. Importantly, HYSPARIN was able to restore normal RSJ patterning and promote rooting competence by suppressing the typical double collet (ectopic RSJ) phenotype observed in the *sec15b* exocyst mutant (Janková Drdová et al., 2019)) and causing AR formation along the hypocotyl in both *sec15b* and wild type. These results demonstrating how intricate connections between vesicle trafficking, hormone control, and environmental stimuli orchestrate root–shoot junction plasticity and AR formation.

Acknowledgement.

The work was supported by the projects CSF/GAČR 25-18138S and TowArds Next GENeration Crops, reg. no. CZ.02.01.01/00/22_008/00 04581, ERDF Johannes Amos Comenius program.

COMPREHENSIVE TECHNIQUES FOR MICROSCOPIC OBSERVATIONS OF FUNGAL ORGANISMS INSIDE THE ROOTS OF WOODY (POPLAR, WILLOW, ...) OR HERBACEOUS (RAPESEED, FLAX, ...) PLANTS

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Key words: biotic root colonization, drought stress, metal toxicity, Solophenyl Flavine staining, tissue bleaching

Root tissues are in tight contact with the soil environment. Both drought stress and metal toxicity in soil may strongly influence the overall plant survival. However, most of the plants are facing environmental issues with intimate coexistence with fungal organisms. Endophytic or mycorrhizal associations may significantly influence the capacity to withstand stress and modify the obtained scientific results. The presence of such associations in root tissues of woody or herbaceous plants is, however, not easy to confirm or evaluate. Here we present a simple protocol for easy and fast staining of fungal organisms in case study on roots of willow and poplar trees. Using effective bleaching technique accompanied by Solophenyl Flavine dye staining allows the fluorescent visualization of fungal structures, both inside or outside of the fine soil-originating roots. Nevertheless, the potential of the protocol could be extended also to other tree species or even to herbaceous plant samples.

Acknowledgement

This work was supported by APVV-23-0318 grant scheme.

ALTERATIONS IN THE PROTEOMIC PROFILES OF ARABIDOPSIS ROOTS TRIGGERED BY SODIUM CHLORIDE STRESS ARE EXACERBATED BY IMPAIRED FUNCTION OF SYNAPTOTAGMIN 1

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Key words: *Arabidopsis*, cytoskeleton, sodium chloride stress, SYNAPTOTAGMIN 1, UHPLC-MS

The *Arabidopsis* SYNAPTOTAGMIN 1 (AtSYT1) is known to play a crucial role in the plant's response to various environmental stressors, including salinity, extreme temperatures, and mechanical damage, by maintaining plasma membrane integrity. In this study, we investigated how sodium chloride stress impacts the proteomic profile in the roots of *Arabidopsis* and whether a mutation in the AtSYT1 gene amplifies these changes. We utilized the T-DNA insertion allele *atsyt1-2*, which has been frequently employed in previous research. Our analysis revealed several pathways affected in both genotypes, including responses to oxidative stress, water deprivation, cytoskeleton organization, proteasome function, and metabolism of glutathione and glucosinolates, as well as lipid transport. While we confirmed changes in the abundance of some proteins known to be influenced by sodium chloride stress, we also identified novel proteins with significantly altered levels. However, when we examined levels of proteins in two genotypes under standard conditions, we found only a small number of proteins with different abundance. This number increased more than twofold when the seedlings were grown on the medium with sodium chloride. We expected to see more pronounced differences between the genotypes due to the ubiquitous and strong expression of the AtSYT1 gene. Therefore, the question arose as to whether the commonly used allele *atsyt1-2* represents a true knock-out line. To address this, we conducted a comprehensive molecular characterization of the allele. Our findings revealed that this allele produces an altered transcript, which exhibits a 75% decrease in abundance compared to the wild type. Sequence analysis suggests that the altered transcript may encode a modified protein with a changed C2B domain at its C-terminus, potentially allowing it to retain functional properties. We propose that *syt1-2* is a weak allele and recommend generating a true knock-out allele for this important gene using advanced techniques such as CRISPR/Cas9.

Acknowledgement

Funding: APVV-23-0463.

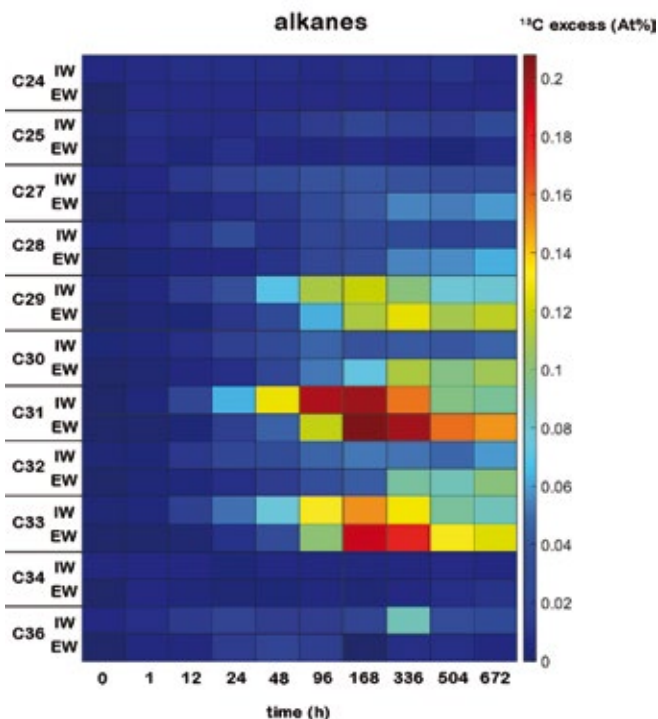
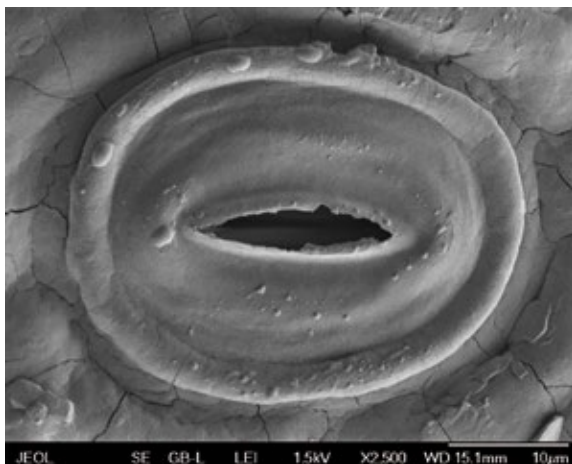
CUTICLE WAX BUT NOT MATRIX IS REGENERATED IN MATURE EVERGREEN LEAVES AND EPICUTICULAR WAX REMOVAL HAS NO EFFECT ON THE RATE OF NEW WAX DEPOSITION

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Key words: cuticle wax, photosynthesis, plant cuticle regeneration, stable isotopes

The contribution summarizes two of our recent related works (one already published). The first introduces a method for monitoring cuticle renewal using ^{13}C labelling. It also shows that new wax is deposited during development in the cuticle of both young and mature leaves of *Clusia rosea* to a comparable extent, while the matrix is renewed only in growing leaves. We also demonstrate that the adhesive technique for wax collection (collodion) does not cause damage to the leaf and can therefore be used for regeneration studies on living plants. The second study focuses on another evergreen plant - cherry laurel (*Prunus laurocerasus*). It also confirms that new wax is deposited to fully mature (overwintering) leaves. A new finding is that the removal of epicuticular wax does not affect the rate of synthesis and deposition of new wax. An interesting finding is that the turnover time varies greatly between different chemical groups of waxes. While the turnover of dominant alkanes is relatively fast (halftime: days to several weeks), pentacyclic triterpenoids (there is a very high concentration of ursolic acid in the cuticle of many *Rosaceae* species) hardly change at all. We are trying to discuss these results in ecophysiological contexts.



PHLOEM-SPECIFIC LOCALIZATION OF SYNAPTOTAGMIN 4 IN ARABIDOPSIS AND ITS POTENTIAL ROLE IN ABIOTIC STRESS RESPONSES

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Key words: abiotic stresses, localization, phloem, sieve elements, Synaptotagmin

The Arabidopsis genome encodes a small family of synaptotagmin homologs, which are calcium-dependent lipid-binding family proteins that have essential roles in several stress conditions. Our study focused on the characterization of SYNAPTOTAGMIN 4 (SYT4), a previously uncharacterized member of the synaptotagmin family. Quantitative GUS fluorometry showed that SYT4 promoter activity was highest in roots and lowest in leaves, which was further confirmed by qPCR analysis. Several factors, such as plant growth regulators, salt, mannitol, sucrose, polyethylene glycol and cold, significantly influenced the promoter activities in roots and upper-ground parts of seedlings. GUS histochemical analysis revealed SYT4 promoter activity in phloem in all organs. It was predominantly active in sieve element precursors and differentiating sieve elements. Correspondingly, the SYT4-GFP fusion protein was also observed in the phloem cells and was particularly abundant in sieve elements precursors. Localization studies indicated that the SYT4 protein initially formed a cytoplasmic network, but as sieve tube differentiation progressed, it gradually deposited at the cell periphery and ultimately disappeared from mature sieve elements. Using the photoconvertible fluorescence protein Dendra2, we demonstrated that SYT4 was abundantly synthesized in developing protophloem meristematic cells. However, protein synthesis ceases as protophloem elements mature, while reduced degradation allows the protein to persist temporarily during differentiation. In addition to its phloem-specific localization, the fusion protein was observed in the stem cell niche of shoots and roots as early as the late heart stage of embryogenesis. In phenotypic analysis, the mutant allele with a T-DNA insertion disrupting the C2B protein domain exhibited increased sensitivity to auxins, osmotic stress, salicylic acid, sodium chloride, and sucrose deprivation in the root growth assay. These findings suggest that SYT4 may play a significant role in phloem and meristem development, as well as in responses to abiotic stresses.

Acknowledgement

This work was funded by APPV-16-0398, APVV-23-0463, & IM-2021-23.

INTER-PROVENANCE VARIABILITY IN DROUGHT RESPONSE OF TREES

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Key words: assisted migration, drought stress, stomatal regulation, xylem vulnerability

European forests face growing threats from prolonged droughts and increasing temperatures. As shifts in precipitation patterns continue, they impose intense selective pressures on tree populations, with potential long-term consequences for forest stability. One proposed climate adaptation strategy is assisted migration (the relocation of seeds or seedlings) from warmer, drier regions to areas where they may be better adapted to future conditions. We examined how tree populations from different geographic origins (provenances) respond to drought stress. Using data from parallel European provenance trials, we focused on key physiological traits: stomatal regulation and xylem function. Our methods included evaluation of stomatal size and density, gas exchange, and assessments of vulnerability to xylem embolism. Results revealed considerable

variability among provenances. Trees from drier climates generally exhibited a faster stomatal response due to smaller guard cells and certain xylem traits that reduced the risk of hydraulic failure. However, short-term acclimation to local conditions often played an even greater role than long-term adaptation. Notably, stomatal behaviour appeared to be influenced by prior climatic exposure, suggesting a form of environmental memory. It is alarming that even after full stomatal closure, water loss through the leaf cuticle and other tissues continues, and under extreme heat, this increases due to lower cuticular resistance. This is particularly concerning, as it suggests that drought combined with heat stress may severely compromise the plant's ability to conserve water. Overall, our study highlights the complex interplay between adaptation and phenotypic plasticity in shaping drought resilience. These insights are crucial for evaluating the potential and limits of assisted migration and for adaptive forest management under a changing climate.

Acknowledgement

This work was supported by the Slovak Research and Development Agency (APVV-21-0270).

MIKROBIOTA OF PLANTS IN METAL POLLUTED ENVIRONMENTS

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Key words: *Arabidopsis arenosa*, heavy metal stress, plant microbiota, plant adaptation

Soil contamination with heavy metals has substantial ecological consequences, including alterations in soil physicochemical properties and disruption of plant-associated microbiota. Soil and plant-associated microbiota play critical roles in modulating plant and ecosystem responses to abiotic stress, yet their taxonomic composition and ecological dynamics under metal stress conditions remain insufficiently characterized. In this study, *Arabidopsis arenosa* – a pseudo-metallophyte naturally occurring in both polluted and unpolluted environments – is used as a model to investigate how plants shape their associated microbiota in response to environmental heavy metal stress, how soil physicochemical parameters influence microbial assemblages, and which microbial taxa functionally contribute to plant adaptation. *A. arenosa* were collected from populations growing at selected polluted and unpolluted sites in Poland, Slovakia, and Austria. Soil samples were also collected from the same locations to characterize microbial communities and to assess physicochemical properties, including pH (H₂O and CaCl₂), soil water content, organic matter content, water holding capacity, labile phosphorus (P), nitrogen (N), and concentrations of selected heavy metals. Microbial DNA was extracted from root, leaf, and soil samples and subjected to next-generation sequencing targeting bacterial (16S rRNA: V5 - V7 regions) and fungal (ITS1) markers to characterize microbial communities. Comparative analysis revealed differences in microbiota composition between plants growing in contaminated and uncontaminated environments. These compositional shifts were strongly associated with variation in soil physicochemical properties. Bacterial and fungal taxa potentially involved in plant stress tolerance were identified and will be further investigated in controlled experiments. Conducting controlled reconstruction experiments will enable functional testing of selected microbial taxa under metal stress conditions. This integrative approach, combining microbial community profiling with controlled experimental reconstruction, is expected to yield detailed insights into the structure and function of plant-microbiota-soil interactions in metal polluted environments

Acknowledgement

The research was funded under grant 2023/49/B/NZ9/01904 from the National Science Centre in Poland

THE OVERLOOKED SIGNAL: REVEALING THE ROLE OF DIHYDROZEATIN IN PLANT REPRODUCTION

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Key words: cytokinin metabolism, dihydrozeatin, plant reproduction, seed development, zeatin reductase

Cytokinins are essential plant hormones that play a key role in regulating almost all aspects of plant growth and development, including reproduction. While the major forms of isoprenoid cytokinins, such as *trans*-zeatin and isopentenyladenine, have been extensively studied, much less is known about dihydrozeatin (DHZ) – a structurally distinct isoprenoid cytokinin characterized by a reduced side chain.

DHZ has been consistently observed to accumulate in plant reproductive structures, such as flowers, fruits, and seeds, across various species. Its levels often peak during critical developmental stages, including floral bud opening, seed filling, maturation and desiccation, when the levels of *trans*-zeatin decline. Despite its confirmed biological activity and clear association with reproductive tissues, both the biosynthetic origin and developmental function of DHZ remain largely unexplored. My research is focused on elucidating the biosynthesis of DHZ and its role in plant reproductive development, with a particular focus on seed formation and maturation. To achieve this, cytokinin profiling using UHPLC-MS/MS was conducted in dry seeds of several crop species to identify developmental stages associated with DHZ accumulation. Several candidate oxidoreductase genes – putative zeatin reductases – were found to be upregulated during DHZ accumulation. Their enzymatic activity was tested via transient expression in *Nicotiana benthamiana*. In a complementary experiment, we confirmed that *Arabidopsis thaliana* is capable of DHZ production when cultured on medium supplemented with *trans*-zeatin. These results support a model in which DHZ is synthesized via enzymatic reduction of *trans*-zeatin during key reproductive transitions. By expanding our understanding of DHZ biosynthesis and function, this research contributes to a more complete view of cytokinin-mediated regulation in plant reproduction. In the long term, uncovering DHZ function could inform new strategies for improving seed quality and yield stability in crops facing variable environmental conditions.

Acknowledgement

The work was supported from the project TowArds Next GENeration Crops, reg. no. CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes Amos Comenius.

ADAPTIVE PROTEOMIC SHIFTS IN BREAD WHEAT SUGGEST MECHANISMS OF DROUGHT TOLERANCE DURING FLOWERING

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Key words: contrasting cultivars, oxidative stress, *Triticum aestivum*, water shortage, yield quality

Water scarcity during anthesis is a critical constraint for bread wheat yield. This study compared the physiological and proteomic responses of two cultivars—drought-tolerant and drought-sensitive—under moderate transient drought during flowering. Measurements of photosynthetic efficiency, water status, and oxidative stress markers revealed that the sensitive cultivar suffered more severe declines in photosynthesis and water retention. In contrast, the tolerant cultivar activated protective mechanisms such as enhanced photorespiration, earlier increased superoxide dismutase activity, and better

maintenance of photosynthesis. Proteomic analysis showed that the tolerant cultivar rapidly adjusted its protein profile early during drought, while the sensitive one exhibited delayed and minimal changes. By the end of the drought, both cultivars showed significant proteomic shifts, more extensive in the sensitive genotype. After rewatering, differences between treated and control plants diminished but remained detectable, especially in the sensitive cultivar. The results suggest that greater proteomic plasticity and early activation of stress-response pathways contribute to the superior drought resilience of the tolerant genotype. Future work will explore redox-related post-translational modifications and their roles in stress adaptation during drought and recovery.

Acknowledgement

This study was supported by the Slovak Research and Development Agency project W-MVP-24-0368 and the National Academy of Sciences of Ukraine grant 0125U001842.

SILVER FIR NEEDLE FUNCTIONAL TRAITS AND OPTICAL PROPERTIES RELATED TO STAND MICROCLIMATE

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Key words: forest health, light microclimate, needle reflectance

Under the ongoing climate change bringing recurrent droughts, heat waves and subsequent pest outbreaks there is a call for more resilient forests than Norway spruce monocultures. Silver fir (*Abies alba* Mill.) appears as a promising candidate species to mixed stands and has been reintroduced into the Czech forests already. However, as a shade requiring species, silver fir young cultures face challenges if the shelter of adult trees was logged due to bark beetle outbreak. On the other hand, in shaded water enriched stands the occurrence of Herpotrichia needle browning caused by a complex of pathogenic fungi can occur.

The aim of the present study is to assess acclimation of photosynthetic apparatus to the different stand light microclimate using chlorophyll fluorescence and assess the physiological status of young silver fir using needle functional traits (pigment, soluble phenolics and proline content, leaf mass per area - LMA). Needle reflectance was related to functional traits and PLSR models for trait retrieval were trained. Additionally, the potential of optical detection of Herpotrichia needle browning was tested.

The preliminary results from two clear cut and two shelter-wood stands will be presented. The maximum quantum yield of PSII showed that silver fir well acclimated to open stands. Needle mass per area, pigment and soluble phenolic contents were more affected by stand light microclimate than water availability. PLSR models for LMA, chlorophyll, water and phenolics content were trained successfully ($R^2 > 0.6$). The adaptability of young silver fir to changing stand conditions will be validated over wide range of light and water availability stand conditions.

Acknowledgement

Funding: The National Agency of Agricultural Research of the Czech Republic, Project QL24010275.

IDENTIFICATION OF THE PUTATIVE PATHWAY OF SALICYLATE BIOSYNTHESIS BY FUNGUS LEPTOSPHAERIA MACULANS DURING INTERACTION WITH THE HOST PLANT

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Key words: *Brassica napus*; Filamentous fungi; *Leptosphaeria maculans*; Salicylic acid; transcriptomics

Leptosphaeria maculans is one of the most destructive pathogens of economically important crop oilseed rape, *Brassica napus*. *L. maculans* is spread by ascospores, causing lesions in leaves at early stages and ending up with stem canker. Upon colonization *L. maculans* produces a range of secondary metabolites to manipulate and evade host plant immunity. Plants have a repertoire of defense mechanisms against fungal invasion, a particular role among which plays salicylic acid (SA). Infection by *L. maculans* triggers SA biosynthesis in plant tissues, which is indispensable for establishing immunity, and regulating programmed cell death. Brassicaceae have 2 major pathways of SA synthesis known as PAL and ICS. PAL pathway is named after enzyme Phenylalanine Ammonia-Lyase which converts phenylalanine to trans-cinnamic acid then to benzoic acid, whose hydroxylation results in SA. ICS (isochorismate synthase) pathway starts in the chloroplasts by converting chorismate derived from shikimate pathway into isochorismate via ICS (isochorismate synthase), which is then transported into cytosol. Some of *L. maculans* effector proteins are known to have reductive effect on SA synthesis, while the fungus was able to accumulate and secrete SA *in vitro*. We hypothesized that *Lm* may also possess a functional biosynthetic machinery and use it during plant colonization. Through ortholog profiling and transcriptomic analysis of a time-course experiment, we have identified 5 *L. maculans* genes potentially involved in pathogenesis and SA-synthesis, 2 genes being orthologous to plant genes encoding key proteins involved in ICS and PAL pathways, the rest being orthologous to genes responsible for bacterial SA synthesis. The *in-silico* data are now being validated by qRT-qPCR with candidate-gene-based primers coupled with measurement of SA levels across compatible-incompatible interactions and *in vitro* fungal cultivation. Discovering the mechanism and role of SA biosynthesis in plant pathogenic fungi will open a new chapter in our understanding of plant-fungi interaction and plant protection.

Acknowledgement

This work is supported by project TowArds Next GENERation Crops, reg. no. CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes Amos Comenius.

NOVEL MODULATORS OF PLANT PLANAR POLARITY

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Key words: gravitropism; membrane domains; planar polarity; root development; trichoblasts

Plant cells' ability to assume planar polarity has been of interest for decades, however limited breakthroughs were achieved due to lack of mutants exhibiting a true loss of planar polarity without pleiotropic defects resulting in embryo lethality. Without a clear method to selectively interfere with planar polarity, the main factors, collectively referred to as the Unknown Polarity Cue (UPC), remain unidentified. The UPC could be a plasma membrane protein(s), a property of the cell wall, or an unknown compound. We generated a new plant line which induces significant and unprecedented root hair displacement, along with other phenotypes consistent with the disruption of the UPC or UPC signalling pathway. The primary objective is to identify the mechanisms disrupted in this transgenic line. Such an approach provides a unique method to advance our understanding of planar polarity and has the potential to revitalise the stagnating field.

Acknowledgement

Project funded by the European Union under the Horizon Europe WIDERA program.

EFFECT OF FLAVOHEMOPROTEIN INHIBITORS ON NITRIC OXIDE IN BARLEY ROOT TIPS

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Key words: Flavohemoprotein inhibitors, NO catabolism, NO emission, Plasma membrane electron transport chain

Nitric oxide (NO) is a key signaling molecule in plants that regulates developmental processes and stress responses. The level of NO in plant tissue depends on the developmental stage and environmental conditions; as well as on production, metabolism and scavenging. Some proteins can be involved in the production of NO, but also in its detoxification. Therefore, we analyzed NO emissions from barley root tips and possible mechanisms of NO catabolism using flavohemoprotein inhibitors, such as azide, cyanide, diphenyliodonium and dicumarol, an inhibitor of the plasma membrane electron transport chain. The roots of barley seedlings were used for experiments, three days after germination. NO emission from the root tips was measured using cell-impermeable fluorescent probes DAF-FM and DAR-4 M, and in contrast, a cell-permeable probe DAR-4 M-AM was used to localize NO. The presented results indicate that the NO consumption activity of root tip cells plays an important role in regulating NO levels in barley root tips. The addition of azide, cyanide, diphenyliodonium, and dicumarol increased not only the amount of NO released into the incubation medium, but also inside the root cells. The applied inhibitors of flavohemoproteins and plasma membrane electron transport chain had an inhibitory effect on NO consumption in the barley root tip cells.

Acknowledgement

This work was supported by the Grant Agency VEGA, project No. 2/0059/24.

THE EVALUATION OF SILICON APPLICATION ON THE GROWTH OF TWO AMARANTH CULTIVARS UNDER SALT STRESS

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Key words: amaranth, morphological parameters, salinity, silicon

Plant growth in saline soils is adversely affected in multiple ways, including reduced biomass, stunted height and decreased yield due to salt stress. The global expansion of salt-affected areas poses a serious threat to future food security. Therefore, there is growing interest among researchers and farmers in identifying crop species that not only tolerate saline conditions but also respond positively to beneficial substances applied as fertilisers. Grain amaranth is recognised for its remarkable adaptability and ability to thrive in degraded soils. In Slovakia, several amaranth cultivars have been bred for inclusion in the food fund of Central Europe. However, their tolerance to salinity and the potential ameliorative effects of silicon (Si) have not yet been thoroughly investigated.

This study aimed to evaluate the tolerance levels to salinity and Si in the first Slovak amaranth cultivar and its parental line. We assessed the morphological responses that occurred after the application of sodium chloride (NaCl) and examined the effect of Si applied as a foliar spray during the 7th and 8th weeks of growth. Morphological traits measured included root and shoot length, as well as fresh and dry biomass.

The most pronounced impact of NaCl was observed on root parameters. Salinity substantially increased the fresh and dry weight of roots in both cultivars and enhanced root length in the parental cultivar. Although the Si application did not influence root biomass, the water content in the roots of salt-stressed amaranth was considerably higher following Si supplementation. In the Slovak cultivar, foliar Si application led to substantial improvements in shoot length, biomass, and water content. In contrast, the parental cultivar showed improvements in these parameters only under salinity stress, except for shoot dry weight, which increased notably after Si application on plants grown in non-saline conditions.

Acknowledgement

This work was supported by the Scientific Grant Agency VEGA, grant number 2/0013/22, and COST Action CA22144.

THE APPLICATION OF NEW MOLECULAR MARKERS IN PEA BREEDING PROGRAMME

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Key words: CAPS, breeding, molecular markers, SNP, pea

Molecular genetics methods enable the selection of suitable breeding materials and thus accelerate the breeding process. As part of the previous project TN01000062 (TAČR 2018-2022), a genome-wide GWAS analysis of pea was performed with the aim of detecting regions of the genome controlling selected traits using 376 SNP markers in 564 genotypes of the genus *Pisum* covering the variability of selected agronomic traits and seed quality traits. These SNPs were subsequently used to design PCR markers for marker-assisted selection to streamline and accelerate the breeding process and to create qualitatively new, competitive varieties of field and garden peas.

Based on the reference sequence and adjacent sequences of SNPs, CAPS (Cleaved Amplified Polymorphic Sequences) markers were designed for easy and fast molecular identification of the reference/alternative alleles in the sample. The next step in testing was to verify the effectiveness of these molecular markers and confirm the association of individual alleles with a specific phenotype on a wider set of samples. Simultaneously with the development of molecular markers, phenotyping of the collection of genetic resources was carried out, targeting resistance traits to pathogens of viral and fungal diseases of pea. Based on association analysis and evaluation of marker segregation and phenotype, the markers are validated and submitted for inclusion in resistance breeding programs. The result of using the markers are functional samples, pea lines, which are described according to the descriptor and stored in the collection of genetic resources in the Czech GRIN database. These genotypes are intended for breeding of new pea varieties with the desired characteristics and with the proven resistance to diseases causing the greatest yield losses.

Acknowledgement

This work was supported by the Technological Agency of the Czech Republic (TAČR), Programme TREND, project No. FW10010461.

DIFFERENTIAL RESPONSE OF MAIZE ROOT CATEGORIES TO ARSENIC TOXICITY: THE ROLE OF SILICON IN LIGNIFICATION AND ANTIOXIDANT ACTIVITY

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Key words: arsenic, lignification, oxidation stress, root categories, silicon

Arsenic (As) is one of the most toxic environmental contaminants, severely affecting plant growth and metabolism through the induction of oxidative stress and disruption of essential physiological processes. This study investigates the responses of three root categories (main, adventitious, and nodal) in two maize hybrids (Tweeter and Luciana) to two levels of arsenic exposure (75 μM and 150 μM) and the mitigating effect of silicon (Si) application, with a specific focus on lignification and antioxidant enzyme activity. Three-way ANOVA revealed that treatment was the most significant factor affecting the measured parameters, with varying responses across hybrids and root types. Arsenic exposure notably reduced root length and guaiacol peroxidase (G-POX) activity, particularly in adventitious and nodal roots, while tyrosine ammonia-lyase (TAL) activity peaked under high As concentrations. The addition of Si partially alleviated these negative effects, especially in nodal roots of Tweeter. Lignin and soluble phenolics (GAE) content were most affected in adventitious roots, with strong correlations between G-POX, PAL (phenylalanine ammonia-lyase), and GAE, indicating activation of the phenylpropanoid pathway as a defense mechanism. Principal component analysis (PCA) distinctly separated As-treated roots from control and Si-treated samples, confirming a coordinated antioxidant and lignification response under As stress.

The results demonstrate root-type-specific responses to As toxicity and suggest a selective, though not universal, protective effect of Si in mitigating arsenic-induced stress. These findings underscore the importance of considering root system complexity and genotype variability in phytotoxicity and stress amelioration studies.

Acknowledgement

The recent work was financially supported by the Slovak Research and Development Agency under contract Nr. APVV-17-0164, and VEGA1/0472/22.

ELICITED *HYPERICUM PERFORATUM* SHOOT CULTURES: A MODEL FOR STUDYING GENES INVOLVED IN HYPERICIN BIOSYNTHESIS

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Key words: elicitation, inhibition, cytochrome P450, phenolics

The genus *Hypericum* is a valuable source of bioactive anthraquinones (AQs) and naphthodianthrones such as photodynamic pigments hypericins, whose incompletely understood biosynthesis limits their biotechnological production. A key step in the biosynthetic pathway leading to naphthodianthrones is the dimerization of two AQ monomers, potentially mediated by cytochrome P450. This enzyme family is known to catalyze radical coupling reactions in plants and is therefore proposed to facilitate the formation of dimeric AQs and flavonoids. Modulation of hypericin biosynthesis using both elicitors and enzyme inhibitors provides a useful platform for studying the regulatory mechanisms underlying the biosynthesis of dimeric AQs.

This study aims to develop *Hypericum perforatum* shoot culture systems with markedly enhanced or suppressed biosynthesis of dimeric phenolic compounds, with a focus on naphthodianthrones and skyrin-derived metabolites. Application of the elicitor chitosan at 10 mg/L increased production of total hypericins by more than 2.5-fold and of the putative precursor emodin by nearly 3-fold. Although the application of proadifen—a non-specific cytochrome P450 inhibitor—caused a slight decrease in AQ content, especially skyrin-derived metabolites (2-fold decrease), its inhibitory effect on the biosynthesis of hypericin and selected flavonols remained ambiguous. Application of clotrimazole stimulated the biosynthesis of hypericins, emodin, flavonoids and chlorogenic acid, with the most pronounced effect observed at a concentration of 25 μM . The inhibitor solvent, DMSO, exhibited mild elicitor activity at low concentrations but had toxic effects and adversely affected plant growth and metabolism at higher concentrations.



These experimental systems provide a basis for comparative transcriptomic analysis aimed at identifying genes encoding enzymes involved in key steps of naphthodianthrone biosynthetic pathway—such as the proposed cytochrome P450—therefore improving our understanding of hypericin biosynthesis and helping to overcome limitations in its potential biotechnological production.

Acknowledgement

This work was supported by VEGA 1/0546/22 and KEGA 015UPJŠ-4/2024.

ROOT BORDER CELL PRODUCTION OF JAPANESE RICE UNDER SALT STRESS

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Key words: Japanese rice, root border cells, salt stress

Root border cells (RBCs) at the root tip act as a biological barrier, facilitating root growth through soil while protecting the root meristem and mitigating salinity stress, which can harm plant growth, photosynthesis, and protein synthesis, thereby reducing crop yield and quality. This study analyzed RBC formation in Japanese rice (*Oryza sativa* L. ssp. *japonica* cv. Koshihikari). Seeds soaked in water for 24 hours, drained, and incubated in darkness produced the highest number of RBCs, with germination occurring within 4–6 days. Under salinity stress, RBC production appeared to decline at NaCl concentrations above 60 mM compared to the control and 10 mM NaCl treatments. RBCs exhibited three distinct shapes: spherical (in the root cap), rectangular (in the division zone), and elongated (in elongation and differentiation zones). Despite limited research on RBCs in monocots like rice, this study highlights their potential role in salinity stress tolerance. Given the economic importance of Koshihikari rice in Thailand and globally, these findings provide a foundation for further research into improving salinity stress tolerance in this crucial crop.

THE ROLE OF GIBBERELLINS IN THE RESPONSE TO OSMOTIC STRESS

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Key words: biosensor, drought, gibberellin, osmotic stress

Drought is considered as one of the most prevalent and damaging stresses, which influences germination, plant development and crop production. Due to the challenges of studying the effect of drought on roots in the soil environment, we currently use osmotic stress to impose water restriction *in vitro*.

Phytohormones play an essential role in early seedling development and its adaptation to stress. We are interested in the involvement of the growth hormone gibberellin (GA) and its interaction with abscisic acid (ABA) in the stress response. GA is

an important hormone which promotes germination, growth of developing tissues as well as flowering. In addition, in response to stress GA plays a major role in the balance between growth and survival, for example in the redistribution of growth between leaves to roots that occurs in response to water limitation. ABA acts primarily to control stomatal behaviour and modifying root architecture to enhance adaptation.

We are using *Arabidopsis thaliana* seedlings to better understand the involvement of GA signaling in the early response to osmotic stress and in the communication between roots and shoots. The stress is induced *in vitro* using polyethyleneglycol and we are monitoring its effect on expression of GA and ABA metabolism genes using qRT-PCR and gene reporters, while the contribution of the genes to the stress response is investigated using metabolism mutants. To determine the effect on GA content and distribution with high spatial resolution we are using the Gibberellin Perception Sensor (GPS2).

Acknowledgement

Funding: IGA_PrF_2025_019; European Regional Developmental Fund to project “Towards Next Generation Crops” No. CZ.02.01.01/00/22_008/0004581(TANGENC).

DO AQUATIC PLANTS FROM CHORNOBYL ZONE RETAIN THE CHRONIC IONIZING RADIATION STRESS-INDUCED MEMORY?

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Key words: DNA damage, epigenetic changes, methyltransferases, proteome, radionuclide contamination

Ionizing radiation creates free radicals, damaging cellular macromolecules. This stress factor triggers multiple signaling cascades in plants, inducing biochemical and metabolic changes. Notably, plants may retain the memory of stress exposure as epigenetic marks inherited by the next generation. As a result, if the progeny is again irradiated, plants could be more tolerant. Our study focused on the plausible mechanisms involved in maintaining the memory of chronic irradiation exposure in a wild aquatic plant (common reed—*Phragmites australis*). Reed seeds were collected from radionuclide-contaminated and clean sites within the infamous Chernobyl zone. We confirmed an equal ploidy level in samples from all locations by counting 48 chromosomes—a tetraploid set. We demonstrated higher DNA damage in the reed seedlings originating from contaminated lakes. After the second challenging exposure of reed seeds to chronic ionizing radiation in the laboratory, we quantified the expression of DNA methyltransferase-related genes. We failed to detect a clear induction or repression pattern. Furthermore, no apparent difference was observed in the lignin content. Follow-up experiments will include: (i) proteome profiling, (ii) quantification of specific free radicals and total antioxidant capacity, and (iii) detection of carbonylated proteins with orthogonal methods. Finally, we will immunolocalize selected differentially accumulated and carbonylated proteins within leaf tissues.

Acknowledgement

The study was supported by the projects APVV-20-0545 and VEGA 2/0106/22.

CONFRONTATION OF GRAIN AMARANTH WITH ABIOTIC STRESS

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Key words: amaranth, heavy metal, histochemistry, morphology, stress

Amaranth (*Amaranthus* spp.) is considered an agronomically important representative of pseudocereals. Moreover, this plant possesses potential traits that make it an attractive model for studying plant responses to negative effects of environmental pollution caused by abiotic stressors including heavy metals, salinity and extreme temperatures, as it can thrive under various harsh conditions. Our research focuses on understanding and exploring the responses of grain amaranth (*Amaranthus cruentus* cv. Pribina) to heavy metal stress under hydroponic conditions¹. We investigated how lead (Pb, non-essential metal) and two essential metals - zinc (Zn) and manganese (Mn) - affect plant performance, growth tolerance indices and root morphology of amaranth. In addition, the accumulation of the tested metals applied in different concentrations over 14 days, was analysed. The spatial localisation of Zn within the amaranth root tissues was demonstrated by histochemical staining at light and fluorescent levels. We found that all metal treatments led to a reduction in plant biomass and to alterations of several root morphological parameters. In particular, Pb and Zn treatments were associated with the most significant reductions in root and total plant biomass, along with a corresponding decrease in growth tolerance indices. The accumulation of the tested metals was higher in the amaranth roots than in the shoots, and the translocation factor values were below 0.5. Zinc accumulation was increased in root tips, developing lateral root primordia, and vascular root tissues compared to the control plants. Our results showed that cv. Pribina exhibits different responses to applied heavy metal stressors. [1] Hunková et al. (2024) A comparative analysis of heavy metal stress responses in different grain amaranth cultivars. *Plant Stress* 14, 100619. ISSN 2667-064X.

Acknowledgement

The work was supported by the Operational program Integrated Infrastructure within the project: Demand-driven research for the sustainable and innovative food, Drive4SIFood 313011V336, cofinanced by the European Regional Development Fund; by Scientific Grant Agency VEGA – grant no. 2/0013/22 and by COST action CA22144.

CULTIVATION OF HYDROPONIC LETTUCE IN PLASMA-ACTIVATED WATER: EFFECTS ON GROWTH, PIGMENTATION, AND SENSORY QUALITY

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Key words: *Lactuca sativa*, phenols, pigments, plasma-activated water, RONS

Cold atmospheric plasma generates multiple reactive oxygen and nitrogen species (RONS), which dissolve in water to form plasma-activated water (PAW), resulting in its subsequent chemical modifications. Due to the presence of RONS, PAW shows considerable potential in agriculture, as these species act as signaling molecules in plant metabolic pathways and enhance nutrient availability. This study evaluates the effects of PAW produced via a transient spark (TS) discharge system on the growth and physiological responses of hydroponically cultivated lettuce (*Lactuca sativa*). Four experimental treatments were established: (1) a control group grown in ½ Hoagland's nutrient solution; (2) plants from seeds primed in PAW for 1.5 hours, then transferred to Hoagland's nutrient solution; (3) plants from seeds primed in PAW and subsequently cultivated in PAW supplemented with ½ Hoagland's nutrient solution; and (4) plants cultivated solely in PAW enriched with ½ Hoagland's nutrients. Plant development was monitored over a 10-week period. At harvest, central rosette leaves (3–4 per plant) were analyzed for pigment content (chlorophylls and carotenoids), as well as pheophytin and phenolic compound concentrations. A sensory evaluation involving 15 participants assessed visual appearance, aroma, flavor, and texture. The PAW treatment showed significantly higher levels of *chlorophyll a* and phenolic compounds, consistent with sensory data indicating this variant as the greenest. However, it exhibited the lowest fresh weight and smallest leaf head size. In contrast, PAW + priming significantly increased biomass and head size, yielding the largest plants among all treatments and was rated the

most palatable. Additionally, this treatment displayed the highest *chlorophyll a+b* activity, indicating enhanced photosynthetic performance.

Acknowledgement

Funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V03-00033 EnvAdvice and Slovak Research and Development Agency APVV-22-0247.

DECODING PHYTOHORMONES AT HIGH RESOLUTION: MAPPING METABOLITE DYNAMICS IN ROOT TIP

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Key words: auxin, cytokinin, FACS, LC-MS, metabolism

Phytohormones are key regulators of plant growth, development, and stress responses, yet their precise roles often remain elusive due to the challenges associated with their detection. These chemically diverse compounds are present in extremely low concentrations, often in the pmol to fmol range per gram of fresh weight, making their detection in plant extracts highly challenging. However, breakthroughs in analytical instrumentation, coupled with the development of micro-extraction and purification techniques, now enable phytohormone analysis at the milligram scale. This progress allows us to move beyond whole-organ studies and investigate hormone distribution at the cellular and even organellar levels.

In our research, we employ fluorescence-activated cell sorting (FACS) to isolate fluorescently labelled cell populations from the root tip of *Arabidopsis thaliana*. Phytohormones are subsequently extracted from these sorted populations, and their metabolic profiles are analysed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Employing these techniques, we would like to generate a high-resolution map of phytohormones within different cell types in the root tip.

Although previous studies have explored this topic, they have either used a limited number of fluorescently labelled *A. thaliana* lines or focused on a single metabolite. Furthermore, these measurements were time and plant consuming, as each sample required hundreds of thousands of cells due to the LC-MS/MS limits of detection. Therefore, we are currently developing cutting-edge analytical strategies to dramatically reduce the number of cells required for analysis by optimizing purification and analytical methods. This will minimize plant material consumption and make phytohormone measurements at the single-cell type level more feasible for routine experiments and should lead to the development of a detailed phytohormonal map in the plant root tip.

Acknowledgement

This work was supported by the project TowArds Next GENERation Crops, of the ERDF Programme Johannes Amos Comenius [grant number CZ.02.01.01/00/22_008/0004581] and by the European Research Council (ERC) under the Horizon Europe research and innovation programme (Grant No. 101166880, STARMORPH – Unravelling Spatio-temporal Auxin Intracellular Redistribution for Morphogenesis).

METABOLIC PROFILING OF PHYTOSTEROID COMPOUNDS IN SELECTED PTERIDOPHYTE SPECIES

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Key words: Brassinosteroids, *Pteridophytes*, Solid-phase extraction, UHPLC-MS/MS

Pteridophytes are among the earliest plants to develop vascular tissue. Their life cycle includes both gametophyte and sporophyte phases, each capable of living independently. The sporophyte stage, equipped with a vascular system, shares similarities with higher plants. Additionally, some pteridophytes exhibit heterospory, meaning they produce two distinct types of spores that give rise to either male or female gametophytes. Pteridophytes have been described to contain all the elements necessary for phytohormonal signaling (Wang et al., 2015). However, the specific roles that these hormones play in pteridophyte growth and development remain largely unknown (Biswal and Panigrahi 2021). Our work aimed to determine the brassinosteroid and mammalian sex hormone (progestagen, androgen and estrogen) profiles in selected *pteridophyte* species. These species include both tropical ferns and those found in the Czech Republic. We used a combination of solid-phase extraction (SPE) and ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) to determine the steroid compounds. We believe that the results of this study will elucidate the role of these compounds in regulating the growth and development of pteridophytes, providing new insights into their hormonal signalling pathways.

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METABOLIC STRATEGY SHIFT IN SPRING TRITICALE SEEDLINGS: FROM HETEROTROPHY TO AUTOTROPHY IN THE CONTEXT OF PHYSIOLOGICAL AND MOLECULAR RESPONSES TO DROUGHT AND SHORT-TERM REHYDRATION

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Key words: drought, rehydration, seedling, spring triticale

Following the first four days of development, during which seedlings remain in a heterotrophic phase relying on grain reserves, a metabolic shift toward autotrophic nutrition begins around day six. This transition involves significant physiological changes, including activation of the photosynthetic apparatus and initiation of independent carbon assimilation. Four- and six-day-old seedlings differ not only in trophic status but also in their resilience to water deficit.

The experiment was conducted on spring triticale seedlings (*cv. Milewo*) at these two developmental stages: heterotrophic (4-day-old, drought-tolerant) and autotrophic (6-day-old, drought-sensitive). Plants were subjected to a four-day drought followed by 24 hours of rehydration. Physiological parameters measured included relative water content, osmotic potential, chlorophyll concentration, gas exchange, and photosynthetic performance. Gene expression analyses targeted transcripts related to water status, stress response, and carbohydrate metabolism: *TaLEA1*, *TaSRG6*, HSP, and RuBp.

During drought, heterotrophic seedlings showed partial stomatal closure and reduced transpiration, resulting in a moderate decline in photosynthesis, less severe than in autotrophic seedlings. After rehydration, only heterotrophic seedlings maintained reduced photosynthetic rates, indicating delayed recovery. Both seedling types experienced significant leaf water content reduction under drought, with autotrophic seedlings showing greater dehydration. Chlorophyll content and membrane stability declined only in autotrophic seedlings and did not recover post-rehydration, reflecting sustained stress. Gene expression revealed a marked increase in *TaLEA1* and *TaSRG6* transcripts in autotrophic seedlings, suggesting their role as markers of drought sensitivity and recovery. In contrast, changes in HSP and RuBp expression were similar in both seedling phases throughout drought and rehydration.

Acknowledgement

Participation in the conference financed from the financial resources of the IFR PAN own research fund.

ADVANCED LIGHT-SHEET AND SUPER-RESOLUTION MICROSCOPY IN ALFALFA RESEARCH

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Key words: alfalfa, immunolocalization protocol, light-sheet fluorescence microscopy, plant-microbe interactions, super-resolution imaging

Medicago sativa (alfalfa) attracts the scientific interest due to its ability to fix atmospheric nitrogen in symbiosis with beneficial soil bacteria in root nodules, providing thus extraordinary biological and agronomical potential. Indispensable in this respect are microscopy approaches usable in biotechnologically oriented crop research that will allow detailed investigation of crops anatomical, structural and physiological characteristics. However, crop samples are robust for imaging at conventional microscopy platforms and sample preparation is, therefore, highly challenging. We have developed two innovative methods for advanced crops microscopic imaging. The first one is significantly improved immunolabeling protocols well-adapted for super-resolution imaging of alfalfa roots (Tichá et al., 2020; Hrbáčková et al., 2021). The second one aims the volumetric imaging by light-sheet fluorescence microscopy (LSFM) allowing to study early symbiotic interaction of alfalfa with beneficial soil bacterium *Ensifer meliloti* on both fixed and living samples (Hlaváčková et al., 2023). In this respect, LSFM is essential to secure unique live-cell imaging conditions for alfalfa-*E. meliloti* interaction that are highly compromised in conventional microscopy systems. These innovative approaches open up new opportunities for studying plant-microbe interactions in real time and for the long term, which is indispensable for full understanding of the symbiotic nodulation process and its biological mechanism.

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Acknowledgement

This work was supported by ERDF project “VIP UP: Development of educational infrastructure and innovative approaches to teaching at Palacký University in Olomouc”, grant number CZ.02.02.01/00/23_023/0009111 and by Palacký University Olomouc grant IGA_PrF_2025_020 awarded to OŠ.

INVESTIGATING THE IMPACT OF HEAT STRESS ON FEMALE GAMETOPHYTE DEVELOPMENT AND SYNERGID CELL FUNCTION IN *ARABIDOPSIS THALIANA*

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Key words: female gametophyte development, heat stress, kinases, ROS, synergid cell

Successful fertilization of the ovule is, among others, dependent on the synergid cells, which are releasing chemoattractants to guide the pollen tube to the ovule. In my PhD project, I will investigate the premature degeneration of the synergid cells during heat stress on a genetic and molecular level. I hypothesize that this premature degeneration is due to premature activation of the programmed cell death pathway involving a protein phosphorylation cascade and resulting in ROS-production. Based on this, I am assessing the involvement and function of several serine/threonine kinases and doing this by phenotyping the ovule structure and the synergid cell function in the corresponding mutants under heat stress condition. Subsequently, I will find the targets of the kinases under investigation by conducting phosphoproteomic assays. In addition, I am also monitoring the impact of heat stress to the female gametophyte (FG) development using various biosensors and FG markers. With my PhD project, I aim to identify involved pathways ensuring synergid cell fitness and function during heat stress as well as to define the main impact of heat stress on the FG development.

Acknowledgement

Plant Sciences Core Facility of CEITEC Masaryk University and the core facility CELLIM supported by the Czech-BioImaging large RI project (LM2023050 funded by MEYS CR) is gratefully acknowledged for the obtaining of the scientific data presented this presentation.

This research is supported by OP JAK TowArds Next GENeration Crops (TANGENC) reg. no. CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes Amos Comenius.

FUNGI INFECTING ROOTS AND STEM BASES IN WINTER WHEAT (*TRITICUM AESTIVUM* L.) IN SLOVAKIA

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Key words: *Fusarium*, necrosis of stem bases, *Phaeosphaeria*, root rot

Winter wheat (*Triticum aestivum* L.) is a key crop in Slovakia, significantly impacted by parasitic microscopic fungi. This study aimed to identify the mycobiota associated with the stem bases and roots of winter wheat at the stage of full physiological maturity. Plant samples were collected from 109 locations in wheat-producing districts of Slovakia during 2019–2020. The study utilized classical morphological methods to identify fungal species based on the characteristics of fruiting bodies observed in situ. The mycological analysis revealed a diverse fungal community, with 12 species identified across the samples. These included *Gibberella zeae* (72%), *Lewia infectoria* (51%), *Monographella nivalis* (50%), *Pyrenophora tritici-repentis* (39%), *Fusarium* spp. (39%), *Colletotrichum graminicola* (39%), *Phoma* spp. (32%), *Lophodermium gramineum* (28%), *Phaeosphaeria herpotrichoides* (28%), *Blumeria graminis* (25%), *Gaeumannomyces graminis* (22%), and *Bipolaris sorokiniana* (22%). Fungi from the genus *Fusarium*, particularly *F. graminearum* and *F. culmorum*, were the most prevalent pathogens. These species are responsible for crown and root rot, leading to significant yield losses in wheat production. Other important pathogens, such as *B. sorokiniana*, *M. nivalis*, and *G. graminis*, were identified as agents of stem base rot in winter wheat in Slovakia. The damage caused by these fungi includes reduced germinability, poor seedling emergence, and post-emergence blight. Many of the identified fungal genera persist on wheat residues in soil, serving as a source of inoculum for the next growing season. This study highlights the importance of managing fungal diseases to maintain healthy wheat crops.

Acknowledgement

This work was supported by the R&D project „Molecular-biological approaches in the solution of plant adaptation to climate change and diagnosis of phytopathogens for ecologically acceptable and sustainable agriculture (contract 1131/2024/MPRVSR-930)“.

POWDERY MILDEW AND RUST FUNGI IMPACTING MEDICINAL PLANTS IN SLOVAKIA

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Key words: biodiversity, Erysiphales, fungal pathogens, medicinal herbs, Uredinales

Medicinal plants are key raw materials for the food and pharmaceutical industries. With the growing demand for herbal medicines, the importance of cultivating healthy plant material has increased significantly. Plant-pathogenic fungi, which include a diverse group of organisms, pose significant threats to both agricultural and natural ecosystems. Among these, powdery mildew and rust fungi are among the most harmful pathogens affecting medicinal plants worldwide. Between 2009 and 2025, surveys were conducted across 40 locations in Slovakia to investigate powdery mildew and rust diseases on both wild and cultivated medicinal plants. Over 55 diseased plant samples were collected, and fungal species were identified using classical identification method and morphological analyses. In particular, fungal communities were examined on symptomatic plant tissues, including the identification of fruiting bodies in dead stem parts. Powdery mildew fungi (Erysiphales), which form characteristic white, powdery film on plant surfaces, are obligate biotrophs. In total, 20 medicinal plant samples were identified as infected by powdery mildew, including species from the genera *Golovinomyces* (7 species), *Erysiphe* (6 sp.), *Neoerysiphe* (1 sp.), and *Podosphaera* (4 sp.). Rust fungi (Uredinales) were also studied, with 20 species identified across 22 host genera. These included species from the genera *Coleosporium* (2 sp.), *Phragmidium* (1 sp.), *Puccinia* (13 sp.), *Pucciniastrum* (2 sp.), *Trachyspora* (1 sp.), and *Uromyces* (1 sp.). The distribution and frequency of these species were compared with available literature data. This research underscores the need for continued monitoring of pathogenic fungi and highlights the importance of managing fungal diseases in medicinal plant cultivation.

Acknowledgement

This work was supported by the R&D project „Molecular-biological approaches in the solution of plant adaptation to climate change and diagnosis of phytopathogens for ecologically acceptable and sustainable agriculture (contract 1131/2024/MPRVS-930)“.

IN VITRO CULTURE OF LOTUS (*NELUMBO NUCIFERA*)

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Key words: Shoot apical meristem, Plant stem cell, Embryo, Secondary metabolite

This study aimed to establish an optimized protocol for tissue and cell culture of Lotus (*Nelumbo nucifera* Gaertn.), focusing on aseptic germination, shoot apical meristem culture, and callus induction. Aseptically germinated lotus embryos were used as explants and subjected to six treatments (20 samples/ treatment). The result show that the highest survival rate (85%) was achieved by soaking the explants in 15% (v/v) of Clorox (6% hypochlorite) for 20 minutes. Embryos of lotus were cultured on Murashige and Skoog (MS) basal medium which can enhance the shoot development within 14 days. The sterilized plantlet production was maintained in a plant tissue culture room for 6 weeks before transferring to liquid MS medium supplemented with 0.3 mg/L TDZ and 8 mg/L NAA. After 2 weeks in liquid media, the multiple leaves emerged, and significant leaf and root proliferation was observed during 3–4 weeks. Comparison of callus induction from embryos and meristematic tissue was used of 4-week-old lotus tissues (including leaves and internodes) that cultured on MS medium containing 2 mg/L 2,4-D and 0.1 mg/L TDZ. During callus induction, the lotus leaf tissues expanded within 7–14 days and small calluses formed within 30 days. Callus induction from sterilized embryos was performed on solid MS medium supplemented with 7.5 mg/L NAA and 0.1 mg/L TDZ for 14 days, followed by transfer to solid MS medium containing 2 mg/L 2,4-D and 0.1 mg/L TDZ for 30 days. As a result, a large and prominent embryo-derived callus was observed. For suspension induction, callus aged 6–8 weeks was scraped and cultured in liquid MS medium with 2 mg/L 2,4-D and 0.1

mg/L TDZ for one week and single cells of lotus were found. We will use the calluses to produce a cell suspension culture focused on plant-stem cell and secondary metabolite analysis in the future, aiming for food and drug development as well as a model of aquatic plant cells for gene transformation.

Acknowledgement

This project was supported by University of Phayao and Demonstration School University of Phayao Science Classroom in University Affiliated School (SCIUS).

GENOME-MINING OF LACCASES IN THE ENDOPHYTE *SEPTORIA ASTERICOLA* ISOLATED FROM *HYPERICUM HUMIFUSUM*

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Key words: bioinformatic analysis, fungal endophyte, laccase activity

Laccases are a group of multicopper oxidases capable of oxidizing small phenolic compounds, with high potential for biotechnological and industrial applications. Many plant-associated endophytic fungi are producers of laccases. Genus *Hypericum* comprises representatives producing unique photodynamically active pigments —naphthodianthrones (hypericins) — with the great potential for therapy and diagnostics of oncological diseases. Despite the biosynthetic pathway of hypericin is not completely elucidated, we consider laccase as one of the candidate enzymes catalyzing phenolic oxidative coupling of emodin molecules. Among the endophytes isolated from *Hypericum* spp., *Septoria astericola* showed high laccase activity based on our previous screenings.

In our work, the genome of *S. astericola* isolated from *H. humifusum* foliar tissues was sequenced and *de novo* assembled. Functional annotation revealed 14 genes encoding for candidate multicopper oxidases, which were divided into different groups based on the phylogenetic analysis and inspection of conserved protein domains. Five oxidases were classified as laccases: three extracellular, one transmembrane and one anchored to plasma membrane. The assessment of total activity of extracellular laccases in a submerged culture using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) showed that the activity increased up to 45th day and then started to decline.

Our findings have highlighted *S. astericola* as a promising candidate for future biotechnological applications, particularly for the production of dimerized phenolics by fungal systems.

Acknowledgement

This work was supported by the Scientific Grant Agency VEGA 1/0546/22 and the Cultural and Educational Grant Agency KEGA 015UPJŠ-4/2024 of the Ministry of Education, Youth and Sports of the Slovak Republic. The authors wish to thank Prof. Eva Čellárová for her valuable contribution to the experimental design.

PEPTIDS IN *MATRICARIA CHAMOMILLA* L.

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Key words: liquid chromatography, *Matricaria chamomilla*, peptid, stress

Matricaria chamomilla is a well-known medicinal plant with anti-inflammatory, antimicrobial, antiviral and antioxidant properties. These effects are due to the presence of secondary substances such as terpenes, flavonoids, coumarins or polyacetylenes. In addition to these, nitrogenous substances - phenylamides or peptides - are also present in chamomile flowers. Thin layer chromatography of the alcohol extract of chamomile flowers with ninhydrin detection indicates the presence of several nitrogenous substances. A combination of liquid column chromatography with HPLC-DAD-MS suggest the presence of another peptide - a dipeptide containing leucine and isoleucine. The role of dipeptides in plants has been linked to plant defenses activated by abiotic or biotic factors, or may be related to nitrogen translocation within the plant organism. The connection of the identified dipeptide within the physiological processes of plant or its therapeutic potential are yet unclear. However, the substance is commonly present and detectable in alcohol extract and therefore warrants attention to investigate its physiological role in more detail.

Acknowledgement

Funding in the realisation of this work and conference paper was provided by The Slovak Grant Agency KEGA under contract No. 003UVLF- 4/2024.

PHOTOSYNTHETIC ACTIVITY AND PROLINE CONTENT OF BLACK PINE NEEDLES INFECTED WITH DOTHISTROMA PINI

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Key words: carotenoids, chlorophylls, Dothistroma needle blight, *Pinus nigra*, proline

Dothistroma pini, the fungus responsible for Dothistroma needle blight in pine trees, has six identified haplotypes (Dp_HAP.1 to Dp_HAP.6) based on variations in the internal transcribed spacer (ITS) region. In this study, we assessed the physiological response of 1–2-year-old needles of black pine (*Pinus nigra*) infected with two Dp_HAP.1 strains (CMW 41493 and M0646; 2×10^6 spores/mL). The experiment was conducted in a growth chamber (Binder KBWF720; 16 h of light at 20 °C, 8 h of dark at 12 °C, 80% humidity) over a period of 25 weeks. Photosynthetic pigment and proline content were measured at four time points (days 1, 49, 98, and 147) in both upper and lower seedling needles. The presence of *D. pini* was confirmed with specific primers PCR for isolate CMW 41493 at the end of the experiment. Results showed that changes in proline and pigment levels depended on the infection phase, needle position, and fungal strain. Early infection resulted in increased proline and decreased pigment content, particularly in the lower needles infected with CMW 41493, compared to the control plant. Proline content proved more responsive to infection than pigment levels, indicating its potential as a sensitive marker of stress. These findings contribute to understanding host-pathogen interactions in *P. nigra* under controlled in vitro conditions.

Acknowledgement

This work was supported by the grant VEGA 2/0034/25 Genetic diversity and pathogenicity of selected fungi that colonize *Pinus* sp. and VEGA 2/0132/22 Impact of climate change on the distribution of selected pathogens of *Pinus* sp. trees

ENDOGENOUS CYTOKININ DYNAMICS IN *A. THALIANA* UNDER VARYING PHOTOPERIODS AND SEASONAL CONDITIONS

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Key words: circadian cycle, CKs, photoperiod, protosynthesis

The effect of photoperiod length and season on endogenous cytokinin content in *Arabidopsis thaliana* is a topic that integrates plant hormonal regulation, photobiology, and environmental physiology. Cytokinins are a class of plant hormones involved in cell division, shoot initiation, leaf senescence, and response to environmental stimuli. Their levels are influenced by both internal developmental cues and external environmental signals, including photoperiod (day length) and seasonal changes. The study monitored circadian variation in photosynthetic performance and cytokinin endogenous levels in *Arabidopsis thaliana* grown under short-day (8h/16h), neutral-day (12h/12h), and long-day (16h/8h) photoperiods during different seasons. Photosynthetic parameters were measured at regular intervals during the day, and plant samples were collected and purified using the StageTip method. Plant hormones were subsequently identified and quantified using UHPLC-MS/MS. The results of this study provide valuable insights into the dynamic regulation of endogenous cytokinin levels in *Arabidopsis thaliana* in response to varying photoperiods and seasonal changes. Our findings indicate that both the length of the photoperiod and seasonal environmental conditions significantly influence cytokinin content, suggesting a complex interplay between light cues and hormonal regulation that governs key physiological processes such as photosynthesis, growth, and senescence. The circadian variation observed in cytokinin levels highlights the plant's ability to adapt to changing environmental conditions, with potential implications for understanding how plants optimize their metabolic processes across different times of day and throughout the year. Further studies are needed to explore the molecular mechanisms underlying these responses and to assess their broader ecological and agricultural relevance.

VETERINARY DRUG RESIDUES IN VEGETABLES: A HIDDEN RISK?

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Key words: accumulation, anthelmintics, manure, vegetable

Anthelmintic drugs are used to control parasitic worms in animals as well as in humans. Although manure is an important source of minerals and organic compounds, it represents a certain risk of spreading veterinary drugs in the farmland and their permeation to human food. In our preceding research, we investigated the interaction between two principal groups of anthelmintics, namely benzimidazoles and macrocyclic lactones, and plant species. These experiments, previously conducted at the laboratory scale, mapped the pathway of these compounds from initial uptake through biotransformation to accumulation. The findings of this study indicate that the presence of these anthelmintics in soil represents a potential risk of their input into the food chain. Recently, our research has focused on conducting experiments in real-world settings and developing analytical methods for the precise detection of albendazole (ABZ) and ivermectin (IVM), as well as their metabolites in vegetables. The experiments were conducted on both conditions: artificially spiked soil with anthelmintics and soil fertilized by manure of animals treated by anthelmintics, respectively. The experiments utilised members of all types of vegetable families - root, leafy, and fruiting. The parent compounds and the metabolically active ABZ metabolite albendazole

sulfoxide (ABZ-SO) were detected in the roots and/or leaves of plants, but not in the fruits. The quantity of these compounds accumulated in plant organs does not pose a significant risk to food consumers. However, it could pose an ecological risk by affecting non-target organisms, such as microbiota or insects.

Acknowledgement

The work was realized under financial support of The Technology Agency of the Czech Republic (SS06020173 -Methods reducing the risks of circulation of veterinary drugs in the environment).

THE LONG CALMODULIN7: NOT JUST A VISITOR, BUT A PLASMA MEMBRANE RESIDENT

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Key words: Ca²⁺ signaling; rapid cell signaling, stress response

Calmodulins (CaMs) are ubiquitous and highly conserved calcium (Ca²⁺) sensor proteins that regulate diverse cellular processes such as morphogenesis, stress responses, and hormone signalling. In *A. thaliana*, all CaM isoforms are considered redundant due to their high sequence similarity and overlapping expression patterns. They predominantly localise to the cytoplasm and are capable of shuttling to the nucleus. However, while CaMs have the propensity for targeting plasma membrane (PM) proteins, none of them have been observed to have an intrinsic PM association. Here we report a CaM7 splicing variant with a C-terminal extension to the canonical form, the 'long Calmodulin 7' (loCaM7), which contains a large polybasic stretch and a prenylation site that is identical to proteins with established PM-localisation, such as AtROP6. By screening transient expressions of fluorescently tagged constructs we confirmed this variant does localise to the PM. Additionally, we have found that loCaM7 is mostly expressed in the root and upregulated in response to various stresses, such as cold. Our aim is to investigate this functionally unexplored splice variant and to elucidate its role within Ca²⁺ signaling in the context of Arabidopsis root stress response, as well as other potential physiological processes.

Acknowledgement

This project is supported by the Czech Science Foundation grant Nr. 25-16449S and by European Union, Horizon Europe, project MOLIPPEC, ID 101087030. We acknowledge the core facility LMH, the BC CAS supported by the MEYS CR (LM 2023050 Czech-Biolmaging).

EFFECTS OF FERMENTED NETTLE EXTRACTS ON ZEA MAYS L. PLANTS DURING DROUGHT STRESS

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Key words: antioxidant properties, biostimulant, glycosidases, proteases

Biostimulants are substances of natural origin that, when applied to plants, increase their growth, nutrient uptake, yield and quality of fruits while also influencing their defense response to stress. Fermented nettles have long been used in traditional

horticulture to increase growth, resistance and yield of garden crops. They present a rich source of nutrients, essential microelements as well as a variety of beneficial microorganisms. However, the exact mechanism of action of this potential biostimulant is still unknown. The fermented nettles (FN) used in this study were firstly characterized in terms of various biochemical parameters: contents of proteins and saccharides and the activity of hydrolytic enzymes related to their degradation, as well as total phenolic compounds and antioxidant power. In the next step, *Zea mays* L. (DKC 3969) plants repeatedly treated with FN, in the form of soil drench, were exposed to drought stress. The FN-treated plants fared better in mitigating the detrimental effects of drought stress than the untreated controls. This was reflected in the contents of leaf pigments, the activity of photosynthetic enzymes and also in the activity of glycosidases. The activities of the antioxidant enzymes (peroxidases, superoxide dismutase, ascorbate peroxidase, and glutathione reductase) which strongly increased in plants exposed to stress, were lowered in FN-treated plants. Together these results indicate that FN-treatment helps to alleviate the negative effects of drought stress by inducing changes in plant antioxidant system, nutrient management and photosynthetic system.

Acknowledgement:

This research was funded by Technology Agency of the Czech Republic, SQ01020132 and by Charles University, Cooperation Program, research area Biochemistry, SVV 260820/2025.

HAIRY ROOT TISSUE CULTURE FOR STUDYING HORMONAL RESPONSES IN BRASSICACEAE

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Key words: auxin, *Brassica napus*, cytokinin, hairy roots, reporter

Hairy root transformation represents a valuable tissue culture system for plant biotechnology in species that are difficult to propagate via standard seed-based methods. Infection by an *Agrobacterium* strain carrying a Root-inducing (*Ri*) plasmid and a subsequent transfer of T-DNA into the plant genome induces the formation of hairy roots at the wounding site of the host plant. Hairy roots can be used to directly analyze a transgene of interest or processed for generation of transgenic plants. Here, we developed and evaluated a genetic reporter system for investigation of auxin and cytokinin in rapeseed (*Brassica napus*) tissues. While these major phytohormones have been widely studied in *Arabidopsis thaliana*, tools for monitoring their spatial signaling activity in crop species remain limited. We constructed two types of GUS-based auxin-responsive reporters driven either by a synthetic pDR5cc or a composite pBnIAA promoters. Each reporter was tested with or without the presence of a 5'UTR. Reporter activity was induced applying exogenous auxin, and GUS expression in hairy roots was visualized via histochemical staining. To insert a new trait, hairy root system can be utilized to re-transform regenerated transgenic plants. To monitor signaling of both auxin and cytokinin in the hairy root tips, we re-transformed the *B. napus* regenerant carrying the TCSv2:3×VENUS cytokinin reporter with *Agrobacterium* strain containing a binary vector encoding the DR5-Tag-BFP auxin reporter. Phytohormone sensitivity of both reporters was assessed by measuring the fluorescence intensity upon exogenous application of auxin and cytokinin.

Acknowledgement

GA23-06140S; the core facility CELLIM supported by MEYS CR (LM2023050 Czech-BioImaging) and Biological Data Management and Analysis Core Facility funded by ELIXIR CZ research infrastructure (MEYS Grant No: LM2023055).

SOME NEW MODELS FOR HYDROTROPISM AND DROUGHT STRESS RESEARCHES IN MOSSES

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Key words: aquaporins, drought stress, epiphylls, moss “canopy”, moss life-forms

Mosses are non-vascular plants, which absorb water as well lose it through their whole surface. Majority of moss species tolerate long-term desiccation retaining the ability to fully recover after hydration. But they grow only under simultaneous action of moisture and light. To save water effectively and thus to prevent their stopping growth when dry, mosses individuals often grow in groups forming so called life-forms. Moss *Plagiomnium rostratum* on the Stradch Mounting, Ukraine was reported as epigeic. On highly illuminated plots it was fully hid under a moss “canopy” mostly consisting of thick wefts of *Rhytidiadelphus triquetrus*. On less illuminated places it grew as a pure life-form or being visible in mixtures with other mosses including *R. triquetrus* (Pundiak, 2023). It is naturally to suppose that such a hiding can take place due to the hydrotropism of *P. rostratum* giving it the ability to avoid drought. *P. rostratum* can serve also as an indicator of water saving effectiveness of a certain moss life-form forming canopy for it. Mosses *Hypnum cupressiforme* and *Brachythecium salebrosum* in arboreta Mlyňany (Slovakia) and Stradch (Ukraine) were reported not only as epigeits, epiphytes and epixyles, but also as epiphylls i.e. grew on leaves and conifers needles (Pundiak, Michalko, 2020; Pundiak, 2021). We can assume that being low sensitive for substrate and its spatial orientation these species formed their life-forms also due to hydrotropism. As arboreta Mlyňany and Stradch are located in relatively dry temperate regions the epiphyllous niche there is very dry comparatively to epiphytic one from where the moss branchlets got from. It gives us new natural models for hydrotropism and drought stress researches. As it is known different aquaporins are key proteins involved as in hydrotropism so in drought stress responses. In mosses aquaporins are poorly investigated and thus are waiting for further investigations.

FOLIAR APPLICATION OF TRICHORMUS SP., A NATIVE ATACAMA DESERT CYANOBACTERIUM, MODULATES OSMOTIC ADJUSTMENT IN SALT-STRESSED TOMATO

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Key words: biostimulant, cyanobacteria, salinity, *Trichormus* sp.

Salinity is a major abiotic stress affecting plant growth, especially in salt-sensitive crops like tomato (*Solanum lycopersicum* L.). Developing sustainable strategies to mitigate its effects is crucial for ensuring agricultural productivity. Cyanobacteria-derived biostimulants have emerged as promising tools to enhance plant tolerance to abiotic stress by modulating physiological and biochemical responses. In this study, we evaluated the effect of foliar applications of a *Trichormus* sp. lysate, cyanobacteria isolated from the Atacama Desert, on tomato cv. Micro-Tom plants subjected to high salinity. The treatment began one day before salt exposure, during the vegetative stage, and continued for three weeks. By the end of the treatment period, plants had entered early flowering. Leaf samples were then collected for biochemical analyses. Results showed a significant positive correlation between the application of the lysate and the accumulation of osmoprotectant sugars such as sucrose and trehalose in the leaf tissue. In contrast, a negative correlation was observed between the treatment and the activity of antioxidant enzymes, including catalase and peroxidase. These findings suggest that foliar application of *Trichormus* sp. lysate may promote salt stress tolerance in tomato through modulation of osmolyte metabolism rather than activation of classical enzymatic antioxidant defenses. The observed accumulation of disaccharides indicates a shift towards osmotic adjustment mechanisms that contribute to maintaining cellular homeostasis under saline conditions. In conclusion, this study provides experimental evidence supporting the role of cyanobacterial

lysates as bioactive agents capable of inducing specific metabolic responses associated with abiotic stress mitigation. These results contribute to our understanding of biostimulant-induced physiological plasticity and support the development of microbial-based strategies for sustainable salinity management in horticultural systems.

Acknowledgement

ANID Doctorado Nacional/2022-21221636, Fondef ID23I10119, ERASMUS+.

METHYL VIOLOGEN-INDUCED CHANGES IN THE *ARABIDOPSIS* PROTEOME IMPLICATE PATELLIN 4 IN OXIDATIVE STRESS RESPONSES

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Key words: IRON SUPEROXIDE DISMUTASE 1, methyl viologen, oxidative stress, PATELLIN4, proteomics

Reactive oxygen species (ROS) accumulation in chloroplasts can cause oxidative stress which rapidly affects protein synthesis, degradation, and protein complex dynamics. In this study, we performed a shotgun quantitative proteomic analysis to monitor the early changes in protein abundances provoked by methyl viologen (MV)-induced oxidative stress in *Arabidopsis thaliana* wild type, *fsd1-1* and *fsd1-2* mutants deficient in *IRON SUPEROXIDE DISMUTASE 1 (FSD1)*. Most of the MV-affected proteins were localized to the chloroplasts in all three lines, while the portion of cytoplasmic proteins was higher in the mutants compared to wild-type ones. Compared with the wild-type, *fsd1* mutants showed significant changes in metabolic protein and chloroplast chaperone levels, together with increased ratio of cytoplasmic, peroxisomal, and mitochondrial proteins. Different responses in proteins involved in the disassembly of photosystem II–light harvesting chlorophyll a/b binding proteins were observed. Moreover, the abundance of PATELLIN 4, a phospholipid-binding protein enriched in stomatal lineage, was decreased in response to methyl viologen. Microscopic analyses proved the decreased GFP-PATL4 fluorescence in epidermal cells of petioles after a 30 min MV treatment. Phenotypic experiments indicated the elevated resistance of *patl4* knock-out mutants to MV. MV treatment caused more pronounced stomatal closure and larger stomata in *patl4* mutants compared to wild-type. PATELLIN 4 is, therefore, a novel protein contributing to the plant response to oxidative stress in chloroplasts.

Acknowledgement

I sincerely thank all co-workers for excellent cooperation and their approach to the topic. I also thank K. Takáčová for excellent technical assistance.

This research was funded by student project IGA_PrF_2025_020 from Palacký University Olomouc.

EFFECT OF WATER DEFICIT AND REHYDRATION ON PHYSIOLOGICAL CHARACTERISTICS OF APPLE TREES

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Key words: apple tree; gas exchange; fluorescence; pigments; water stress

The apple tree (*Malus domestica* Borkh.) is one of the most economically important continental crops worldwide and one of the most consumed fruit species in the world. The area of orchards in the Czech Republic in 2022 was 15 419 ha, of which 5 864 ha were apple orchards. One of the reasons leading to a reduction in the area planted is the occurrence of water deficit (drought), which adversely affects plant growth, survival and limits crop productivity, causing a reduction in fruit yield and quality. The effect of water deficit was monitored under partially controlled conditions of the foliar cover in selected apple genotypes: Gala, Idared, Frosta, B11, HL 53, HL 155-05 in a container experiment, where water deficit was induced for 14 days followed by one week of rehydration. The experiment was carried out at developmental stages 31 BBCH to 67 BBCH. Among the physiological characteristics, pigment content, photosynthesis and transpiration rates, chlorophyll fluorescence were monitored. The results show that the effect of water deficit leads to a decrease in all the observed characteristics. Under the influence of rehydration, all physiological parameters increased, but the values of control plants were not reached. The rehydration period of 7 days is short for apple trees. The results show that the increase in transpiration values during the dry season in the varieties Gala and Frosta makes them unsuitable for drier areas. Idared, on the other hand, is a more plastic variety. The genotypes HL 53, HL 155-05 appear to be promising genotypes with potential for further research.

Acknowledgement

This work was created with the support of the grant project of the Ministry of Agriculture of the Czech Republic - NAZV QK21010200: Breeding of fruit species for abiotic resistance in combination with high content of antioxidant substances in fruits.

THE ROLE OF SILICON SUPPLEMENTATION IN INCREASING PRODUCTIVITY OF OILSEED RAPE - HISTOLOGICAL CHANGES IN LEAF ANATOMY

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Key words: *Brassica napus var napus* L., drought, FT-Raman spectroscopy, leaf gas exchange, orthosilicic acid

Due to their sedentary lifestyle, plants must adapt to the harmful environmental factors to which they are constantly exposed. Silicon is an element naturally occurring in large quantities in the earth's crust, but its absorption by plants is limited. Therefore, silicon had long been overlooked in the context of plant stress protection. However, it has been shown that silicon stimulates the growth and development of plants, and also alleviates the effects of abiotic and biotic stresses, which is particularly important in the era of changing climate. Oilseed rape is a popular crop worldwide, but it is not a good silicon accumulator. However, it has been known for some time that silicon supplementation also brings benefits to oilseed rape, although the mechanism of its action is still unknown. In the presented studies, spring oilseed rape plants of the Markus variety were used. Plants growing in well-watered conditions or drought were supplemented with silicon in the form of orthosilicic acid. Photosynthetic rate, the general chemical composition of leaves and histological analyses of plants samples were studied. It was shown silicon supplementation enhanced the photosynthetic rate significantly which might further indicate higher plant productivity in both well-watered conditions and drought. Although the analysis of the chemical composition of leaves did not show significant changes between control and silicon-supplemented plants, significant differences were observed in the reconstruction of tissues both in well-watered and droughted conditions, which were translated into increased transport of assimilates and water and were associated with intra- and inter-cellular signal transduction (eg. plastid stomules). In summary, silicon supplementation in oilseed rape led to anatomical modifications of plant organs, which in turn influenced the physicochemical properties of tissues. These structural changes may play a key role in improving water management, particularly under drought stress.

SUBCELLULAR LOCALIZATION PATTERNS OF ANNEXIN 1 IN ARABIDOPSIS

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Key words: annexins, development, root hairs, subcellular localization

Annexins represent an evolutionary conserved multigene family of proteins capable of binding calcium ions (Ca^{2+}) and membrane phospholipids. The genome of the model plant *Arabidopsis thaliana* comprises eight annexin genes *ANN1-ANN8*, encoding proteins of molecular mass from 32 to 42 kDa. Annexins are expressed throughout the plant body and across various stages of the life cycle. Annexins are involved in wide range of fundamental cellular processes, including signal transduction during plant growth and development, as well as responses to environmental stimuli. Based on their biochemical characteristics - such as Ca^{2+} binding, interaction with phospholipids and F-actin, and their ATP/GTPase activities, annexins are hypothesized to participate in exocytosis, endocytosis, and intracellular vesicular trafficking. ANNEXIN 1 (ANN1) is the most abundant member of protein superfamily of annexins. Advanced microscopy methods enable to investigate tissue-specific expression and subcellular localization patterns of ANN1-GFP during the early developmental stages of *Arabidopsis* seedlings. Light-sheet fluorescence microscopy (LSFM) revealed the preferential localization of ANN1-GFP in trichoblasts and developing root hairs. At the subcellular level, ANN1-GFP was observed in close proximity to the endoplasmic reticulum (ER), surrounding the nuclear envelope, and associated with small spherical structures in the cytoplasm, as revealed by lattice LSFM. Notably, in developed bulges and actively elongating root hairs, the spatial distribution of ANN1-GFP reflected the characteristic tip-focused Ca^{2+} gradient associated with polar growth, suggesting a potential role for ANN1 in the regulation of root hair initiation and tip growth. This study reveals the complex spatiotemporal expression patterns and subcellular localization of ANN1 during post-embryonic development of *Arabidopsis thaliana* (Tichá *et al.*, 2020).

Reference: Tichá *et al.* (2020) *Front. Plant Sci.* 2020;11:1153

Acknowledgement

This work was supported by Palacký University Olomouc (grant Nr. IGA_PrF_2025_020).

PHYSIOLOGICAL BASIS OF SALINITY TOLERANCE: LESSONS FROM PLANTAGO SPECIES

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Key words: carbohydrates, *Plantago*, salinity, sorbitol, stress

Sugar alcohols are key photosynthetic products in some plant groups, facilitating long-distance carbon transport and enhancing stress tolerance, particularly under drought and salinity. Sorbitol, a linear sugar alcohol common in *Plantaginaceae* species, shares a precursor (glucose-6-phosphate) with sucrose. This suggests a finely tuned regulation of carbon partitioning based on the plant's physiological status. While sucrose metabolism is well-studied, the regulation of sugar alcohol pathways remains poorly understood. This project investigates carbohydrate metabolism in selected *Plantago* species with varying stress tolerance. We focus on sorbitol and sucrose distribution under both control and salt stress conditions, monitoring carbohydrate allocation, enzyme activity related to sorbitol metabolism, and related gene expression profiles. Metabolomic data already show distinct sucrose and sorbitol profiles between glycophytic and halophytic species.

Unexpectedly, our research also revealed striking differences in sodium (Na^+) and potassium (K^+) uptake among *Plantago* genotypes. Under salt stress, all species absorb Na^+ , but halophytes accumulate it to much higher levels without apparent

toxicity, indicating efficient ion compartmentalization. Moreover, despite the competition between Na⁺ and K⁺, halophytes tolerate low K⁺ levels. To delve deeper into this, we use TEM-EDS microscopy to identify the location of ions within vacuoles, cytosol, and chloroplasts. Initial results suggest that salt tolerance is not due to reduced Na⁺ uptake but rather to effective intracellular storage. An additional intriguing focus is the presence of unique endodermis with Casparian strips in the vasculature of *Plantago* sp. leaves. The significance of these structures remains unclear, but they may be linked to the genus's life strategies.

These findings deepen our understanding of how sorbitol metabolism and ion compartmentalization contribute to stress tolerance in *Plantago*. The presence of Casparian strips in leaf vasculature may also play a role in these adaptations. Insights gained could inform breeding strategies to improve salt and drought tolerance in sorbitol-producing crops, such as those in the *Rosaceae* family.

Acknowledgement

The work is supported by Charles University Grant Agency – Project 460325.

NEW *IN VITRO* METHODOLOGY FOR GENOTYPIC SCREENING OF TOLERANCE TO OSMOTIC AND SALINITY STRESS USING MARKER GENES *PsCAT1*, *PsDREB1*, *PsDREB2A* AND *PsLEA2* IN PEA (*PISUM SATIVUM* L.)

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Key words: drought and salinity tolerance, expression of related genes, *in vitro* selection, *Pisum sativum*, wild relatives of pea

The aim of the study was to develop a methodology for determining tolerant pea genotypes based on *in vitro* screening using known marker genes associated with osmotic stress: *PsCAT1*, *PsDREB1*, *PsDREB2A* and *PsLEA2*. The screening of 15 pea genotypes revealed significant differences in growth, morphology and marker expression in response to combined PEG6000 and NaCl as a stress factors. The stress tolerance was found in wild relatives of pea, *P. fulvum* and *P. elatius*, and was characterized by differences in the expression of the *PsLEA2* and *PsCAT1* genes.

Acknowledgement:

This work was supported by the Ministry of Agriculture of the Czech Republic, institutional support MZE-RO1023, by the Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Publ. Res. Inst. (the institutional support VUKOZ-IP-00027073) and by the IGA UP Olomouc (PrF-2023-001).

JUVENILE SCOTS PINE RESPONSE TO DROUGHT: PROVENANCE VARIABILITY AND THE POTENTIAL OF OPTICAL METHODS FOR STRESS ASSESSMENT

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Key words: drought stress; chlorophyll fluorescence; Scots pine; spectral reflectance; stress memory

As a result of climate change, forest ecosystems are facing more frequent and intense drought episodes, increasing the demands on the resilience of tree species. Phenotypic plasticity and the ability to adapt to changing environment play a key role for tree survival. The recurrence of drought raises the issue of 'stress memory', a phenomenon whereby previous exposure to stress influences subsequent physiological responses. This phenomenon is still understudied in woody plants. Scots pine (*Pinus sylvestris*) is a species with high phenotypic plasticity, but locally adapted populations – provenances – may show markedly different responses to water availability.

This study evaluates the complex physiological response of juvenile Scots pine to limited water availability and aims to investigate the memory effect after previous exposure to drought, using non-destructive optical methods. Seedlings of four provenances covering the north-south gradient of the species' range (one Norwegian, two Czech and one Turkish) are monitored over two vegetation seasons (2025-2026) in an outdoor container experiment involving nearly 400 individuals. The experiment is designed so that a controlled watering regime can test the effect of previous stress (control-control; dry-dry; control-dry; dry-control). Growth, physiological and optical parameters, including RGB imaging, VIS-NIR reflectance, thermography and chlorophyll fluorescence fast kinetics, are regularly measured on the seedlings. In the first year of the study, we are testing hypotheses related to among provenances variation in response to water deficit and the sensitivity of different optical methods to detect early drought symptoms and recovery. Initial preliminary results from the current first season indicate differences in optical properties, chlorophyll fluorescence dynamics, and phenological phases among provenances, as well as between control and stress treatments.

Acknowledgement

Grant agency UK, project 378625.

XYLOGLUCAN ENDOTRANSGLYCOSYLASES: SUBTLE STRUCTURAL DIFFERENCES WITH SIGNIFICANT IMPACT ON THE PLANT CELL WALL

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Key words: cell wall, mutational analysis, primary structure, structural polysaccharides, xyloglucan endotransglycosylase

Xyloglucan endotransglycosylases (XETs) are major enzymes involved in plant cell wall reconstruction and reorganization during various physiological processes and stress-induced damage. The enzyme's name is derived from the main reaction that they catalyze, i.e., cleavage of structural xyloglucan and transfer of the fragment with its former nonreducing end

to another molecule of xyloglucan. In addition to xyloglucan, another plant structural polysaccharides able to serve as donors or acceptors were found with specific isoenzymes. Both in vivo and in vitro XET activities have been detected using fluorescent acceptors.

Combining methods of biochemistry (enzyme assays) with bioinformatics and computational analysis, we identified amino acid residues potentially responsible for XETs acceptor nonspecificity. The validity of this approach was confirmed by substituting the corresponding residues and performing activity assays on the mutants. Generally, two main amino acid residues were identified as responsible for acceptor nonspecificity, with an additional two residues involved in binding charged acceptors, all located within the XET binding site. The influence of amino acid residues in the extended C-terminus of the XET GH16_20 subfamily was also demonstrated, highlighting the role of basic residues in the reaction between xyloglucan or cellulose and pectin.

Considering that the only 3D structure of XET corresponds to a strictly specific enzyme, our research is currently focused on purifying a form of barley XET that possesses the ability to transfer fragments of cellulose or xyloglucan to various plant cell wall structural poly- and oligosaccharides, with the goal of determining its 3D structure and completing the structure–substrate specificity studies of these enzymes.

Acknowledgement

This work was supported by VEGA grant 2/0162/24.

COMPARATIVE STUDY OF GAS CHROMATOGRAPHY AND PHOTOACOUSTIC DETECTION FOR MEASURING ENDOGENOUS ETHYLENE IN PLANTS

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Key words: ethylene, gas chromatography, photoacoustic detection

We developed and compared gas chromatography (GC-FID) and photoacoustic detection (ETD-300) for measurement of ethylene production in plants. Both of these methods have been used in the past to measure ethylene production in plants, but a detailed comparison of these approaches and an attempt to merge them into a single pair have not yet been published. Our aim was to develop an approach, combining the advantages of both methods. While the use of GC has been proven to be very effective and requiring small amounts of sample gas, photoacoustic detection provides more sensitive measurements. Our approach involves measuring plant fresh mass which is giving us a possibility to determine the endogenous levels per unit fresh plant weight. This allows us to recognize whether the change in ethylene production was due to an increase in plant fresh weight and/or whether the treatment actually affects ethylene production per se. Our approach is also unique in that unlike typical methods, where plants are grown on treated media, we grow them on untreated media and the plant treatment is applied at a precise time period. This allows us to eliminate the effect of the treatment on germination and more accurately simulate the effect of tested compounds under field conditions. Another advantage is that the subsequent use of the combined approach on the same sample allows us to recognize machine errors that would otherwise be attributed to the biological variability. We are also able to save the samples for further hormonal measurements by mass spectrometry. The method has been successfully tested for use with Arabidopsis plants treated with compounds known from the literature to affect ethylene production. Although the machines do not provide numerically identical values, the observed trends are identical and the values are in the same order of magnitude.

Acknowledgement

The work was supported from European Regional Development Fund-Project "SMART Plant Biotechnology for Sustainable Agriculture" (No. CZ.02.01.01/00/23_020/0008497.

FUNCTIONS OF THE EXO84A EXOCYST SUBUNIT IN ARABIDOPSIS POLLEN

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Key words: Arabidopsis, exocyst complex, polarized growth, pollen

Pollen grain germination and pollen tube elongation are crucial biological processes in angiosperm plants that involve polarized secretion. Precise regulation of this process requires the exocyst tethering complex that targets secretory vesicles specifically to the plasma membrane. We focused on the EXO84 exocyst subunit in pollen and found that out of three EXO84 isoform in Arabidopsis EXO84a represents the main isoform functioning in the male gametophyte. EXO84a interacts with EXO70 and SEC15 subunits in the Y2H system similar to EXO84b in the sporophyte. Mutants in EXO84a generate shorter pollen tubes, resulting in a transmission defect of the mutant allele. EXO84a-GFP is localized in growing tips of pollen tubes similar to other exocyst subunits. We conclude that EXO84a is a crucial component of the exocyst complex in Arabidopsis that is required for efficient polarized secretion specifically in pollen.

TOWARDS BIOCONTROL OF BACTERIAL DISEASES: NEW PSEUDOMONAS PHAGE ISOLATION, CHARACTERIZATION, AND GENERATION OF A LIBRARY OF Tn5 MUTANT P. SYRINGAE PV TOMATO

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Key words: bacteriophages, *Pseudomonas syringae*, characterization, mutant library

In the context of reducing the use of hazardous agrochemicals and mitigating bacterial resistance to them, bacteriophages (phages) appear to be promising biocontrol agents for crop protection against bacterioses. We aim to develop a functional phage-based solution to combat bacterioses of tomato. To do so, we have isolated a set of novel phages from the environment, and are now characterizing their properties, mechanisms of interaction with the host, and efficiency against *Pseudomonas syringae* pv. *tomato* (*Pst*) in *Arabidopsis thaliana* model plant. Two new phages have been isolated from pepper fruits (Pap5 and Pap7), which appeared to be stable at a range of environmentally relevant pH and temperatures, and efficient against an array of *Pseudomonas* bacteria *in vitro* and *in planta*. We have developed a method of phage formulation and spray application which enhanced phages persistence on leaves and ensured efficiency when applied before the bacterial inoculation. We are currently investigating the bacterial receptors which are targeted by these phages using Tn5 transposon mutagenesis. A library of mutated *Pst* strains has been prepared and will be exposed to the phages, followed by individual sequencing of the resistant colonies. We aim at identifying the *Pst* genes involved in phage adsorption, which will help us understanding the mechanisms of phage resistance and predict wider host range through bioinformatics comparison. This prediction would help us to ensure treatments efficiency by developing phage mixtures that cover a wide enough spectrum of bacterial receptors, avoiding emergence phage resistance through single mutation.

Acknowledgement

This work is supported by Technological Agency of Czech Republic (TAČR, TQ03000088). IEB Imaging Facility is supported by MEYS, grant LM2023050 „Czech-BioImaging“. The project of generation of the library of *P. syringae* mutants was supported by STSM grant of the COST Action CA22158 MiCropBiomes.

PHD-HD PROTEINS: AN ENIGMATIC PLANT-SPECIFIC TRANSCRIPTION FACTOR FAMILY

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Key words: callus, chromatin, histone reader, meristem, PHD-HD proteins

Transcription factors (TFs) are key regulators of gene expression and plant development. The plant-specific PHD-HD TF family, defined by a plant homeodomain (PHD) and homeodomain (HD) architecture, is represented in *Arabidopsis thaliana* by only two members: HAT3.1 and PRHA. Despite their close phylogenetic relationship, these proteins diverge substantially in molecular features, subnuclear organization, and developmental roles. HAT3.1 forms distinct nuclear condensates that partially colocalize with chromocenters. TurboID-based proximity labeling identified methyl-CpG-binding domain proteins (MBD1/2/4) as top interactors of HAT3.1; these co-localize with HAT3.1 speckles, suggesting formation of functional nuclear complexes. Furthermore, a histone peptide array revealed that the PHD domain of HAT3.1 specifically recognizes H4K20me3, a histone modification with largely unexplored roles in plants. In contrast, PRHA displays diffuse nuclear localization, and its PHD domain lacks histone binding specificity. Promoter-reporter analyses demonstrate that *HAT3.1* is expressed throughout the meristematic zone of the root apical meristem, while *PRHA* expression is confined to initials and pericycle cells. Both genes are upregulated during callus induction and shoot regeneration, indicating a role in cellular reprogramming. Notably, *prha* mutants exhibit accelerated callus formation and enhanced regeneration capacity, suggesting a role in repressing cell proliferation. In summary, our data revealed a pronounced functional divergence within the PHD-HD TF family and pointed to their distinct contributions to chromatin-associated regulation and developmental reprogramming in plants.

Acknowledgement

Supported by LUAUS24277 and TANGENC CZ.02.01.01/00/22_008/0004581.

DO PLANTS USE THE ACTIN CYTOSKELETON IN DNA REPAIR?

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Key words: ARP2/3 complex, arpc5, DNA repair, DSB, HR

DNA double-strand breaks (DSBs) pose a significant threat to the cell, as they can lead to chromosome disintegration and cell death if they are left unrepaired. For this reason, a few distinct repair pathways have evolved, including homology-directed repair pathways such as single-strand annealing (SSA) and homologous recombination (HR). It appears that this complex repair pathway must cooperate with the cytoskeleton, as well as other cellular systems. In animals, it has been shown that the cytoskeletal complex ARP2/3 (which enables de novo nucleation and branching of actin filaments) plays a critical role in HR. However, there is no such evidence of the cytoskeletal role in DNA repair in plants. Therefore, to investigate the possible role of ARP2/3 on DNA repair, we have crossed *Arabidopsis* lines mutated in subunits of the ARP2/3 complex with the fluorescent marker line for HR, RAD54-YFP, allowing us to track and compare the repair pathway in real time in individual cells. To further assess how the loss of ARP2/3 affects

the choice of DNA repair pathway over time at the whole-plant level, we performed a homologous recombination assay in ARP2/3 mutant backgrounds. We found a significant increase in the number of DSBs that are being repaired by HR in individual cells, and also an increase in total repaired lesions by both SSA and HR pathways in the *arpc5* background, one of the ARP2/3 complex subunits. These results indicate the role of ARP2/3 in the maintenance of genome stability or the DNA repair itself; however, further research is still needed.

AFTERMATH OF TRANSIENT DROUGHT ON GRAIN PROTEOME OF WHEAT

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Key words: contrasting cultivars, LC-MS, storage proteins, *Triticum aestivum*, water shortage

Drought stress frequency and intensity have significantly increased globally because of climate change, which has been particularly detrimental to crop productivity, including the widely cultivated bread wheat (*Triticum aestivum*). Stress during the reproductive stage has the most potent effect on yield decline. Herein, we evaluated the impact of moderate drought during flowering on grain quality across 2 contrasting cultivars—the sensitive cultivar Chyhyrynka and the tolerant cultivar Sofiia Kyivska. Proteins were isolated with single-step detergent-assisted extraction, digested with trypsin, and processed with liquid chromatography-mass spectrometry. We quantified 5,433 proteins in mature grains and revealed 728 differentially abundant proteins across genotypes and drought treatment. According to the principal component analysis, genotype contributed more to protein accumulation variance than drought treatment, with a distinct grouping of tolerant and sensitive cultivars. The protein profiling determined that seed storage proteins, such as glutenin, gliadin, cupin type-1, serpin, and globulin isoform 1, accumulated similarly after drought in both cultivars. Furthermore, several proteins involved in metabolic reactions were significantly depleted upon stress. Of note, the total grain yield declined considerably in the sensitive genotype. Next, we will focus on redox proteome alteration in flag leaves under water shortage and subsequent recovery at the reproductive stage as the most critical for yield formation. The discoveries will reveal molecular markers and pathways essential for developing drought-resilient wheat to improve crop yield in the face of progressing climate change.

Acknowledgement

The study was supported by the project VV-MVP-24-0368.

OPTIMIZED ATAC-SEQ LIBRARY PREPARATION FROM FLOWSORTED MAIZE POLLEN NUCLEI HARBORING ACCESSORY B CHROMOSOMES

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Key words: ATAC-seq, B chromosomes, flow cytometry, maize, pollen

Pollen grains play a central role in plant reproduction, delivering the male gametophyte to fertilize egg cells. In angiosperms, pollen typically contains one vegetative nucleus and two sperm nuclei. However, small size, rigid cell wall, and cytoplasmic content of pollen grains pose challenges for isolating nuclei, which complicates genomic analyses. Yet, purified nuclei free from organellar and extranuclear DNA contamination are essential for high-resolution downstream applications such as ATAC-seq. In this study, we optimized nuclei isolation and ATAC-seq library preparation from maize (*Zea mays*) pollen obtained from lines with different genetic backgrounds, focusing on lines carrying B chromosomes — supernumerary, nonessential chromosomes that exhibit non-Mendelian inheritance via mechanism termed chromosome drive. In grasses (Poaceae), B chromosomes undergo nondisjunction during the second pollen mitosis, resulting in their preferential transmission through sperm cells. While typically not beneficial to the host genome, B chromosome accumulation can negatively impact fertility. We used flow cytometry to isolate nuclei from B73 line with and without 2B chromosomes, Mo17 line, and their hybrid Mo17xB73 (MB), also with and without 2B chromosomes. This approach allowed us to examine chromatin dynamics in maize pollen and to explore the genomic impact of selfish genetic elements like B chromosomes. Our results contribute to a deeper understanding of nuclear organization in plant gametes and lay the groundwork for improved epigenomic profiling in reproductive tissues.

EVALUATION OF *POPULUS* SPP. CLONES FOR PHYTOREMEDIATION OF ANTIMONY-CONTAMINATED ENVIRONMENT

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Key words: antimony, antioxidant enzymes, phytoremediation, poplar, stress markers

Antimony (Sb) is a toxic metalloid whose elevated concentrations in the environment are predominantly linked to historical and ongoing mining and metallurgical activities. In certain regions of Slovakia, Sb contamination poses a considerable environmental threat. Phytoremediation, particularly when employing suitable plant species, offers a sustainable and cost-effective strategy for the stabilization or removal of Sb from contaminated sites. Fast-growing trees of the genus *Populus* are considered promising candidates for phytoremediation applications due to their high biomass production and notable tolerance to abiotic stress. In this study, we evaluated the physiological and biochemical responses of three *Populus* spp. clones cultivated under hydroponic conditions in the presence of Sb. The activities of antioxidant enzymes — peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) — were quantified as biomarkers of oxidative stress. In addition, levels of malondialdehyde (MDA) and proline were measured to further assess the extent of stress-induced damage and plant adaptation mechanisms. Our findings revealed significant interclonal variability in response to Sb exposure, indicating that specific *Populus* isolates possess enhanced potential for Sb phytostabilization. These results support the prospective application of selected poplar genotypes in the remediation of antimony-contaminated soils in Slovakia.

Acknowledgement

This work was supported by Slovak Grant Agency VEGA, grant 2/0047/25 and Slovak Research and Development Agency, grant APVV-23-0318. We thank Irena Mravíková for the technical support.

SPRAY-INDUCED GENE SILENCING AS A STRATEGY TO PROTECT *PAPAVER SOMNIFERUM* AGAINST *BOTRYTIS CINEREA* AND *APHIS FABAE*

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Key words: *Papaver somniferum*, *Botrytis cinerea*, Aphids, RNAi, SIGS

The Czech Republic is a global leader in the cultivation of *Papaver somniferum* for food industry, where it holds cultural and culinary significance. However, like other crops, *P. somniferum* is susceptible to various pathogens and pests that negatively impact yield. Among the most common are the fungal pathogen *Botrytis cinerea*, which causes grey mold on foliage, and aphid species such as the poppy aphid (*Aphis fabae*) and the green peach aphid (*Myzus persicae*). Intensive and widespread use of fungicides and pesticides could pose significant risks to the environment and potentially human health- EU has in its „Farm to Fork“ strategy the aim to decrease the usage of chemicals up to 50 %. It brings demand for new more specific and environmentally friendly crop protection strategies. Such strategy represents usage of RNA interference in the approach called Spray-Induced Gene Silencing (SIGS). In SIGS alternatively, synthetic double strand RNAs (dsRNA) are applied topically to the plant surface with the aim to be uptaken by pathogen or pest and silence their essential gene(s). Which should lead to significant decrease of their viability. In our work we established poppy pathosystems with the above-mentioned pathogen and pests under control conditions and we designed and produced dsRNA(s) specifically targeting them. We tested the effect of our dsRNAs on our pathosystems. We believe that RNAi approach represent a promising, species-specific, and eco-conscious tool for protecting *P. somniferum* from biotic stress and once established the pipeline could be used for protecting *P. somniferum* against plethora of pathogens and pests.

Acknowledgement

We would like to thank for financial support from MEYS Inter-Excellence II, Inter-COST project nr. LUC23146.

DETECTION OF PROTEIN S-NITROSATION IN PLANTS: BIOTIN SWITCH VERSUS SNO-RAC METHODS

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Key words: biotin switch; redox modifications, S-nitrosation

Posttranslational modifications of protein cysteines, including oxidation, S-nitrosation and persulfidation, are important mechanisms that regulate protein biological activity and localisation. The most important source of biologically available nitric oxide is apparently S-nitrosoglutathione (GSNO), which is believed to be involved in the control of protein S-nitrosation status. We have tested and optimized the detection method of S-nitrosated protein cysteines known as SNO-RAC (resin-assisted capture of S-nitrosated proteins). We also compared this method to a more commonly used biotin-switch technique (BST). Individual steps of both methods were modified to achieve a higher yield of isolated S-nitrosated proteins. Both methods were experimentally tested using bovine serum albumin as a model protein as well as proteins extracted from tobacco cell culture (*Nicotiana tabacum* cv. Xanthi). The influence of important parameters such as detergent choice, concentrations and volumes of key reagents, the length and the way of incubation of proteins with matrices, was examined. It has been concluded that the BST method shows higher detection sensitivity and easier work with neutravidin matrix, on the other hand, lower price and shorter time of sample processing can be considered as the advantage of SNO-RAC method.

SHEDDING LIGHT ON DROUGHT STRESS: THE ROLE OF PHOTOTROPINS AND ABA IN DROUGHT RESPONSES IN *ARABIDOPSIS THALIANA*

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Key words: Abscisic acid, blue light, drought, phototropins

How do plants balance water conservation and survival under drought stress? Abscisic acid (ABA) is known to induce stomatal closure to minimise water loss, while blue light (BL) induces stomatal opening and affects stomatal movement through phototropins. But could phototropins influence ABA signalling beyond stomatal control, or vice versa? This study investigates their role in drought stress responses using phototropin mutants of *Arabidopsis thaliana*.

In vivo drought stress experiments revealed that mature *phot2* mutant plants are more drought sensitive under irradiation by WL and BL, exhibiting lower ABA levels under WL, but surprisingly increased ABA accumulation under BL. Gene expression analysis suggests that this is related to *BG1* gene involved in ABA deconjugation, together with up-regulation of *NCED3* gene key for ABA biosynthesis, and *RD29b* gene, a drought stress marker.

In vitro assays with polyethylene glycol (PEG) 8000 confirmed that phototropins, principally PHOT2, influence root growth under conditions of drought stress. Furthermore, *in vitro* assays with ABA treated seedlings demonstrated that the *phot2* mutant exhibited a diminished sensitivity to ABA-induced inhibition of root growth, irrespective of the light conditions present. However, ABA metabolism itself remained largely unchanged, suggesting that phototropins - particularly PHOT2 - affect ABA sensitivity rather than biosynthesis.

These findings raise new questions about how plants integrate light and hormone signalling to optimise stress responses.

Acknowledgement

This work was supported by IGA_PrF_2025_019 grant and Visegrad Fellowship 62510023.

ROLE OF BEL1-LIKE TRANSCRIPTION FACTORS IN POTATO TUBERIZATION

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Key words: BEL1-like transcription factors, potato, *Solanum tuberosum*, tuberization

Tuberization, the process in which underground stems (stolons) develop into storage organs (tubers), is a key physiological event directly influencing potato yield. While the effects of environmental cues such as photoperiod, temperature, and nitrogen availability are well documented, the internal mechanisms that translate these signals into developmental outcomes remain not fully understood. Although several important molecules involved in the regulation of tuberization—including BEL1-like transcription factors—have been identified, most knowledge to date comes from studies on the model genotype *Solanum tuberosum* ssp. *andigena*. Given the potato's status as the world's fourth most important food crop, elucidating the molecular control of tuberization in commercial varieties is essential for future breeding strategies. To contribute to this understanding, we employed RNAi and CRISPR/Cas9-induced gene knockout to target *BEL1-like* genes in cultivated potato genotypes. Our findings reveal that key components of the tuberization signaling network function differently in *andigena* and cultivated varieties, pointing to genotype-specific regulatory divergence within *S. tuberosum*.

Acknowledgement

This work was supported by the GAUK project No. 1308119, and by the project TowArds Next GENeration Crops, reg.no. CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes Amos Comenius.

Errata

Plant Biology CS 2025

Preferred section: *Phytohormones and root & shoot development*

Preferred type of contribution: *Poster presentation*

KARRIKINS AS NOVEL PLANT GROWTH REGULATORS: SIGNALING PATHWAYS AND IMPLICATIONS FOR CROP PRODUCTION

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Key words: Karrikinolide (KAR₁), phytohormone crosstalk, seed germination, smoke-compounds, sustainable agriculture
Karrikins are a class of butenolide compounds produced by the combustion of carbohydrates - commonly abundant in plant material - and are typically found in plant-derived smoke and are recognized as potent plant growth regulators with promising applications in enhancing seed germination. Karrikins, especially karrikinolide (KAR₁), were first identified in wild-fire-prone environments and have proven to be highly effective in germination of a variety of crop species, including those that are not inherently exposed to fire-related cues.

The karrikin signaling pathway involves perception through the α/β hydrolase receptor KARRIKIN INSENSITIVE2 (KAI2), which forms complexes with the F-box protein MAX2 to create an SCF E3 ubiquitin ligase complex. The complex targets transcriptional repressor proteins SMAX1 and SMXL2 for degradation, thereby activating downstream gene expression. Recent research suggests karrikins mimic an endogenous butenolide ligand termed "KAI2 ligand", indicating a fundamental signaling pathway in plant development.

Karrikin applications demonstrate significant benefits across major crop categories. In cereals, KAR₁ enhances wheat germination and root development, while improving maize seedling vigor and activating aquaporin genes. Legume responses vary, with species-specific effects observed in soybean under different light conditions. After receiving KAR₁ treatment, horticultural crops such as tomato and celery show increased germination rates and seedling establishment.

Beyond germination, karrikins enhance stress tolerance in crops by improving antioxidant enzyme activities and maintaining redox homeostasis under drought and salt stress conditions. The compounds integrate with multiple phytohormone pathways, particularly gibberellin signaling, creating complex regulatory networks that modulate seed germination responses. Despite promising biological activities, commercial challenges including high synthesis costs and lack of field validation protocols restrict widespread application. Future research should focus on developing cost-effective delivery systems and validating field efficacy in order to maximize karrikins' efficacy in sustainable agriculture.

Acknowledgement

The work is supported by the institutional funding of the Ministry of Science and Higher Education of the Republic of Poland awarded to the University of Agriculture in Kraków.

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České společnosti experimentální biologie rostlin
a Fyziologické sekce Slovenské botanické společnosti

of the Czech Society of Experimental Plant Biology
and the Physiological Section of the Slovak
Botanical Society

2025

ISSN 1213-6670